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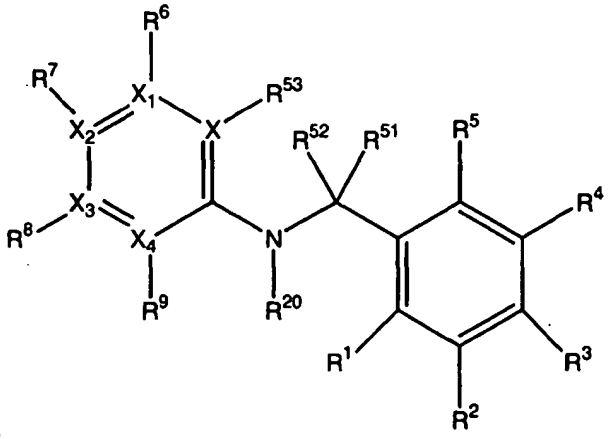
(54) Title: SALICYLAMIDES AS SERINE PROTEASE INHIBITORS



(I)

(57) Abstract: The present invention provides novel compounds of Formula (I), its prodrug forms, or pharmaceutically acceptable salts thereof. The compounds of this invention are inhibitors of serine proteases, Urokinase (uPA), Factor Xa (FXa), and/or Factor VIIa (FVIIa), and have utility as anti cancer agents and/or as anticoagulants for the treatment or prevention of thromboembolic disorders in mammals. The present invention also provides a process for the selective acylation of an amino group.

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SALICYLAMIDES AS SERINE PROTEASE AND FACTOR XA INHIBITORS

FIELD OF INVENTION

The present invention relates to novel serine protease inhibitors.

BACKGROUND OF THE INVENTION

One of the most active areas in cancer research is in the field of proteolytic enzymes and their role in the spread of cancer. One class of proteases that plays a significant role in the progression of cancer are the serine proteases, in particular Urokinase-type plasminogen activator (uPA). Inhibitors of uPA have been postulated to be of therapeutic value in treating cancer. Inhibitors of these serine proteases also tend to be inhibitors of the closely related blood-clotting enzymes. One such blood-clotting enzyme is Factor Xa.

Factor Xa (herein after "FXa"), the converting enzyme of pro-thrombin to thrombin, has emerged as an alternative target (to thrombin) for drug discovery for thromboembolic disorders. A variety of compounds have been developed as potential FXa inhibitors.

Kunitada and Nagahara in Current Pharmaceutical Design, 1996, Vol. 2, No.5, report amidinobenzyl compounds as FXa and thrombin inhibitors. Disclosed in U.S. Patent No. 5,576,343 are aromatic amidine derivatives and salts thereof, as reversible inhibitors of FXa. These compounds comprise amidino substituted indolyl, benzofuranyl, benzothienyl, benzimidazolyl, benzoxazoyl, benzothiazolyl, naphthyl, tetrahydronaphthyl and indanyl groups, attached to a substituted phenyl ring by an alkylene group having from 1 to 4 carbon atoms.

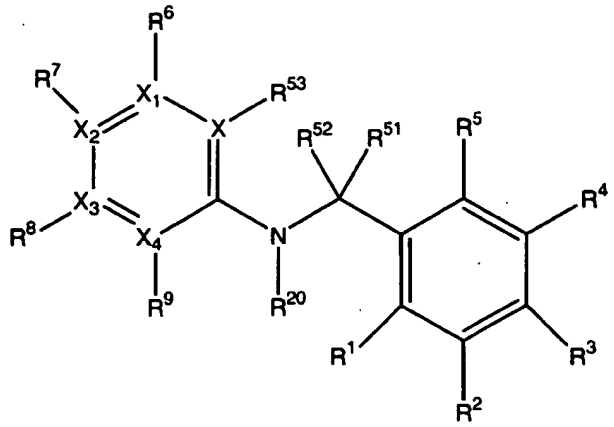
In spite of the above discussed efforts, desirable treatment of cancer and thromboembolic disorders still remains elusive. There is thus a need for new compounds that will be effective in inhibiting serine proteases, such as Urokinase, and blood-clotting enzymes such as FXa. Keeping these needs in mind, the present invention provides novel inhibitors as discussed below.

SUMMARY OF THE INVENTION

Keeping the above discussed needs in mind, the present invention provides novel salicylamides of Formula I as serine protease inhibitors. Included in the present invention are pharmaceutically acceptable salts of compounds of Formula I, a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a compound or a pharmaceutically acceptable salt of a compound of Formula I, a method of treating or preventing a thromboembolic disorder, comprising administering to a patient in need thereof a therapeutically effective amount of a compound of Formula I, and a method for treating cancer in mammals comprising administering a therapeutically effective amount of a compound of Formula I. Also provided by the present invention is a process for selectively acylating an amino group.

DETAILED DESCRIPTION

Provided by the present invention is a compound of Formula I:

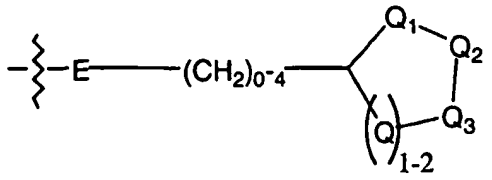


Formula I

its prodrug form or pharmaceutically acceptable salts thereof, wherein:

R^1 represents OH, COOH, COO-C_{1-4} alkyl, CH_2OR^{10}, SO_2-OH, O-SO_2-OH, O-SO_2-OC_{1-4} alkyl, $OP(O)(OH)_2$, or OPO_3C_{1-4} alkyl;

R^2, R^3, R^4, and R^5 independently at each occurrence represent H, SH, OR^{10}, halogen, $COOR^{10}$, $CONR^{11}R^{12}$, optionally substituted aryl, optionally substituted heterocyclyl, C_{4-14} cycloalkyl-C_{1-4} alkyl, C_{1-4} alkyl aryl, optionally substituted C_{1-14} straight chain, branched or cyclo alkyl, $NR^{10}R^{24}$, $(CH_2)_{1-4}$-$NR^{33}R^{34}$, $(CH_2)_{1-4}$-$COOR^{33}$, O-$(CH_2)_{1-3}$-CO-het, O-$(CH_2)_{1-2}$-NH-CO-aryl, O-$(CH_2)_{0-2}$-NR^{10}-CO-$NR^{10}R^{33}$, O-$(CH_2)_{0-2}$-C(O)-$NR^{33}R^{34}$, O-$(CH_2)_{1-4}$-$COOR^{10}$, O-$(CH_2)_{1-3}$-het-R^{32}, O-optionally substituted cycloalkyl, O-$(CH_2)_{1-4}$-NR^{10}-COO-*t*-butyl, O-$(CH_2)_{1-4}$-$NR^{10}R^{33}$, O-$(CH_2)_{1-4}$-NR^{10}-C(O)-C_{0-3}-alkyl-optionally substituted aryl, O-$(CH_2)_{0-6}$-optionally substituted aryl, $(CH_2)_{1-4}$-NH-C(O)O-$(CH_2)_{1-4}$-$PhR^{13}R^{14}$, NO_2, O-$(CH_2)_{0-4}$-C(O)-NH-tetrahydro carboline, SO_3H, $CH(OH)COOR^{10}$, $NR^{10}R^{28}$, O-$(CH_2)_{1-3}$-optionally substituted het, CH_2COOCH_3, CH=CH-$COOCH_3$,



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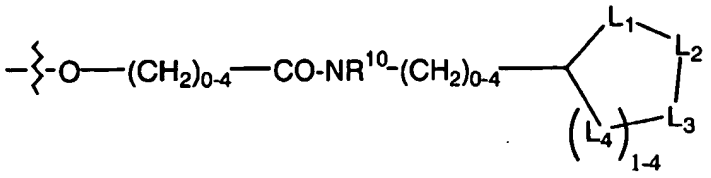
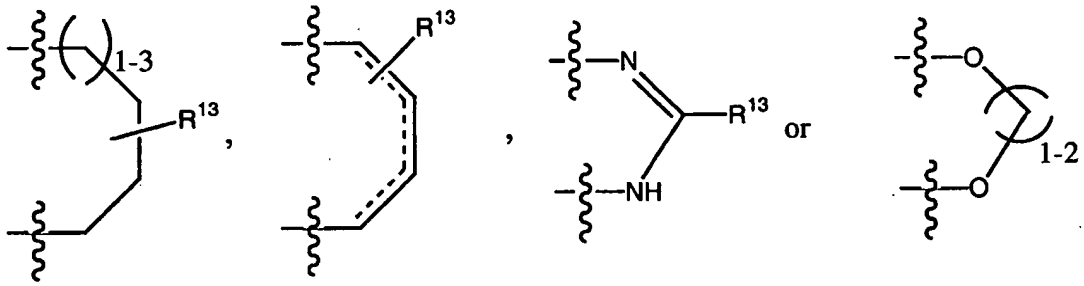


;

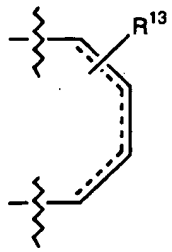
alternatively R^2 and R^3, R^3 and R^4, or R^4 and R^5 taken together form



or ;

R^6, R^9 and R^{53} independently at each occurrence represents H, halogen, cyano, C_{1-4} alkyl, C_{1-4} halogenated alkyl, NO_2, O-aryl or OR^{11};

alternatively R^6 and R^{53} taken together form



;

R^7 and R^8 independently at each occurrence represent OH, CF_3, H, COOH, NO_2, C_{1-4} alkyl, OC_{1-4} alkyl, or O-aryl, halogen, cyano, or a basic group selected from

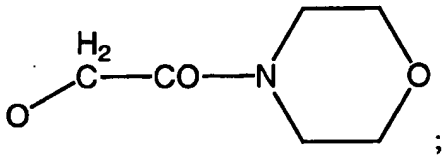
guanidino, $NH(CH=NH)NH_2$, $C(=NH)N(R^{10})_2$, $C(=NH)$-NH-NH_2, $C(=O)N(R^{10})_2$, 2-imidazoline, N-amidinomorpholine, N-amidino piperidine, 4-hydroxy-N-amidino piperidine, N-amidino pyrrolidine, tetrahydro pyrimidine, $C(O)CH_2NH_2$, $C(O)NHCH_2CN$, $NHCH_2CN$, and thiazolidin-3-yl-methylideneamine; with the proviso that only one of R^7 and R^8 represent a basic group;

R^{10} independently at each occurrence represents H, $(CH_2)_{0-2}$-aryl, C_{1-4} halo alkyl, or C_{1-14} straight chain, branched or cyclo alkyl, and alternatively, when one atom is substituted with two R^{10} groups, the atom along with the R^{10} groups can form a five to 10 membered ring structure;

X_1, X_2, X_3 and X_4 independently at each occurrence represent a carbon or a nitrogen atom;

R^{11} and R^{12} independently at each occurrence represent H or C_{1-4} alkyl;

R^{13} represents H, OH, OC_{1-4} alkyl, OAr, OC_{5-10} cycloalkyl, OCH_2CN, $O(CH_2)_{1-2}NH_2$, OCH_2COOH, OCH_2COO-C_{1-4} alkyl or



;

R^{20} represents H or OH;

R^{24} represents R^{10}, $(CH_2)_{1-4}$-optionally substituted aryl, $(CH_2)_{0-4}OR^{10}$, CO-$(CH_2)_{1-2}$-$N(R^{10})_2$, $CO(CH_2)_{1-4}$-OR^{10}, $(CH_2)_{1-4}$-$COOR^{10}$, $(CH_2)_{0-4}$-$N(R^{10})_2$, SO_2R^{10}, COR^{10}, $CON(R^{10})_2$, $(CH_2)_{0-4}$-aryl-$COOR^{10}$, $(CH_2)_{0-4}$-aryl-$N(R^{10})_2$, or $(CH_2)_{1-4}$-het-aryl;

R^{28} represents $(CH_2)_{1-2}$-Ph-O-$(CH_2)_{0-2}$-het-R^{30}, C(O)-het, CH_2-Ph-CH_2-het-$(R^{30})_{1-3}$; $(CH_2)_{1-4}$-cyclohexyl-R^{31}, CH_2-Ph-O-Ph-$(R^{30})_{1-2}$, CH_2-(CH_2OH)-het-R^{30}, CH_2-Ph-O-cycloalkyl-R^{31}, CH_2-het-C(O)-CH_2-het-R^{30}, or CH_2-Ph-O-(CH_2)-O-het-R^{30};

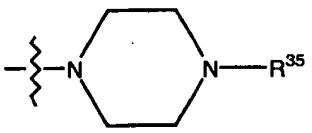
R^{30} represents $SO_2N(R^{10})_2$, H, NHOH, amidino, or $C(=NH)CH_3$;

R^{31} represents R^{30}, amino-amidino, NH-$C(=NH)CH_3$ or R^{10};

R^{32} represents H, C(O)-CH_2-NH_2, or C(O)-$CH(CH(CH_3)_2)$-NH_2;

R^{33} and R^{34} independently at each occurrence represent R^{10}, $(CH_2)_{0-4}$-Ar, optionally substituted aryl, $(CH_2)_{0-4}$ optionally substituted heteroaryl, $(CH_2)_{1-4}$-CN, $(CH_2)_{1-4}$-$N(R^{10})_2$, $(CH_2)_{1-4}$-OH, $(CH_2)_{1-4}$-SO_2-$N(R^{10})_2$;

alternatively, R^{33} and R^{34} along with the nitrogen atom that they are attached to forms a 4 to 14 atom ring structure selected from tetrahydro-1H-carboline; 6,7-Dialkoxyoxy-2-substituted 1,2,3,4-tetrahydro-isoquinoline,



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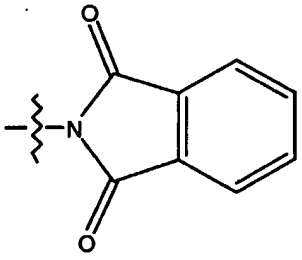


;

R^{35} represents R^{10}, SO_2-R^{10}, COR^{10}, or $CONHR^{10}$;

E represents a bond, $S(O)_{0-2}$, O or NR^{10};

Q, Q^1, Q^2, Q^3, L^1, L^2, L^3 and L^4 independently at each occurrence represent N-natural or unnatural amino acid side chain, CHR^{10}, O, NH, $S(O)_{0-2}$, N-C(O)-NHR^{10}, SO_2-$N(R^{10})_2$, N-C(O)-NH-$(CH_2)_{1-4}$-R^{26}, NR^{10}, N-heteroaryl, N-C(=NH)-NHR^{10}, or N-$C(=NH)C_{1-4}$ alkyl;

R^{26} represents OH, NH_2, or SH;

R^{51} and R^{52} independently represent COOH, CH_2OH, CH_2COOH, COOR, CH_2COOR, alkyl or $CO\text{-}NH_2$; alternatively

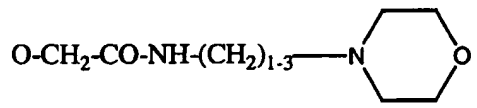
R^{51} and R^{52} taken together represent =O, =S, $=CH_2$ or $=NR^{10}$;

R^{53} represents H, halogen, cyano, $C_{1\text{-}4}$ alkyl, $C_{1\text{-}4}$ halogenated alkyl, NO_2, O-aryl or OR^{11};

with the proviso that at least two of X_1, X_2, X_3 and X_4 represent a carbon atom, and when any of X_1, X_2, X_3 and X_4 represent a nitrogen atom the corresponding substituent does not exist.

In a preferred embodiment of the present invention is provided a compound of Formula I wherein, R^1 represents OH or COOH; R^{20} represents H; R^{51} and R^{52} taken together form =O; and X_1, X_2, X_3, and X_4 represent C. Another preferred embodiment provides a compound wherein, R^2 represents halo, H, NH-CO-Ph, *i*-propyl, OH, OCH_3, OC_2H_5, CH(OH)COOH, O-*I*-propyl, SO_3H, NH_2, $CH(OH)COOC_{1\text{-}2}$ alkyl, CH_3, NO_2 or Ph;

R^3 represents H, OH, NH_2 $OC_{1\text{-}4}$ alkyl, $C_{1\text{-}4}$ alkyl, $NHCH_3$, $O\text{-}(CH_2)_{1\text{-}3}\text{-}OCO\text{-}C_{1\text{-}2}$ alkyl, $NH\text{-}C(O)C_{1\text{-}2}$ alkyl, $O\text{-}(CH_2)_{1\text{-}2}\text{-}CO\text{-}NH_2$, Ph, $NHCOCF_3$, $N{=}CH\text{-}N(CH_3)_2$, $O\text{-}CH_2\text{-}CO\text{-}NH\text{-}(CH_2)_{1\text{-}3}\text{-}Ph$,



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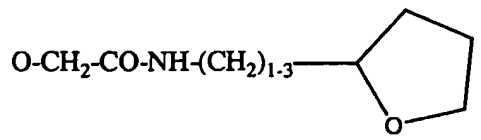

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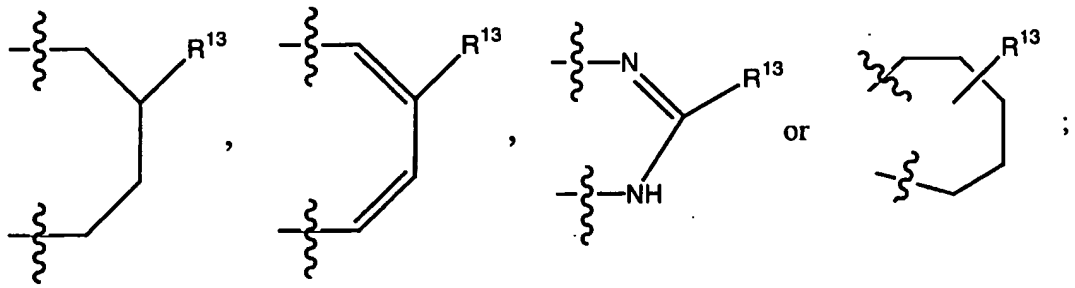
R^4 represents H, $C_{1\text{-}4}$ alkyl, halogen, *i*-propyl, OH, NH_2 3-nitro-phen-1-yl, NH-CO-CH_3, $CH_2\text{-}NH\text{-}(CH_2)_3\text{-}Ph$, 2,4-difluoro-phen-1-yl, $NHCOCF_3$, benzo[1,3]dioxol-5-yl,

4-Carbamimidoyl-phenylazo, 3-Hydroxy-4-carboxyl-phenylsulfanyl; 1,3-Dioxo-indan-2-yl, or toluene-4-sulfonylamino;

R^5 represents H or OH;

alternatively, R^2 and R^3, R^3 and R^4, or R^4 and R^5 can be taken together to form



;

R^6 represents H;

R^7 represents C(=NH)-NH_2 or NH-C(=NH)-NH_2;

R^8 represents H or halogen; and

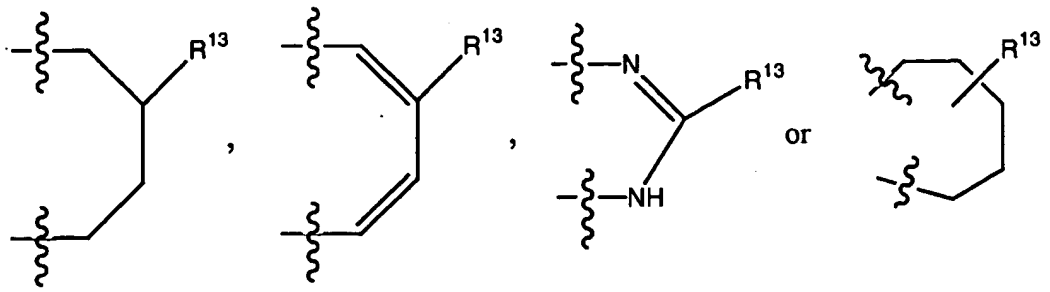
R^9 represents H.

A further preferred embodiment provides a compound wherein, R^2 represents halo, H, NH-CO-Ph, *i*-propyl, OH, CH_3, or NO_2;

R^3 represents H, OH, NH_2 OC_{1-2} alkyl, C_{1-4} alkyl, O-$(CH_2)_{1-3}$-OCO-C_{1-2} alkyl, NH-C(O)CH_3, O-CH_2-CO-NH_2, Ph, $NHCOCF_3$, N=CH-$N(CH_3)_2$, O-CH_2-CO-NH-$(CH_2)_2$-Ph;

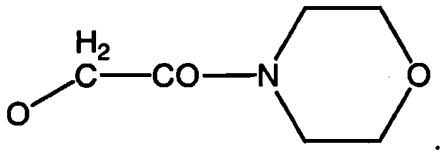
R^4 represents H, CH_3, methoxy, halogen, *i*-propyl, 3-nitro-phen-1-yl, $NHCOCF_3$, benzo[1,3]dioxol-5-yl, $NHCOCH_3$, 4-Carbamimidoyl-phenylazo, 3-Hydroxy-4-carboxyl-phenylsulfanyl or 1,3-Dioxo-indan-2-yl;

alternatively, R^2 and R^3, R^3 and R^4, or R^4 and R^5 can be taken together to form



or ; and

R^{13} represents C_{1-2} alkyl, OH, $O(CH_2)_{1-2}$-NH_2, H, or



Particularly preferred compounds of the present invention are:

N-(4-Carbamimidoyl-phenyl)-2-hydroxy-3-iodo-5-methyl-benzamide;

3,5-Dibromo-N-(4-carbamimidoyl-phenyl)-2,4-dihydroxy-benzamide;

5-Bromo-N-(4-carbamimidoyl-phenyl)-2,4-dihydroxy-3-iodo-benzamide;

3-Hydroxy-naphthalene-2-carboxylic acid (6-guanidino-pyridin-3-yl)-amide; and

3-Hydroxy-7-methoxy-naphthalene-2-carboxylic acid (4-guanidino-phenyl)-amide.

Another aspect of the present invention provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of (i) a compound; or (ii) a pharmaceutically acceptable salt of a compound of Formula I. Also provided by the present invention is a method of treating or preventing a thromboembolic disorder, comprising administering to a patient in need thereof a therapeutically effective amount of a compound of Formula I, or a pharmaceutically acceptable salt thereof.

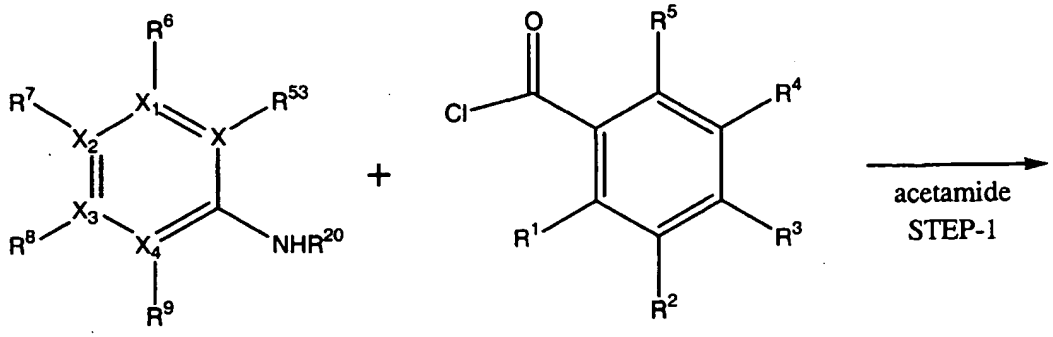
In yet another aspect of the present invention is provided a process for selectively acylating an amino group, said process comprising treating a molecule comprising an amino group with an acylating agent in the presence of an acetamide to yield a compound with an acylated amino group. A preferred embodiment provides a process wherein the amino group is selectively acylated in the presence of another acylatable group. Yet another preferred embodiment provides a process wherein the acylatable group is selected from an optionally substituted amino ketone, alkyl amidino, alkyl guanidino, $C(=NH)NH\text{-}NH_2$, aryl-$(CH_2)_{0\text{-}4}$-NHR^{10}, amidino and guanidino; the acylating agent comprises an acid halide group; and wherein the acetamide is an alkyl or dialkyl acetamide.

A further preferred embodiment provides a process wherein the acetamide is selected from a group consisting of DMA, diethyl acetamide, dimethyl propionamide, diethyl propionamide and N-methylpyrrolidinone; the process is carried out at a temperature ranging from about 25°C to about 50°C; and wherein the acylating agent is a protected salicylic acid chloride selected from acetic acid 2-chlorocarbonyl-phenyl ester and 2-benzyloxy-benzoyl chloride.

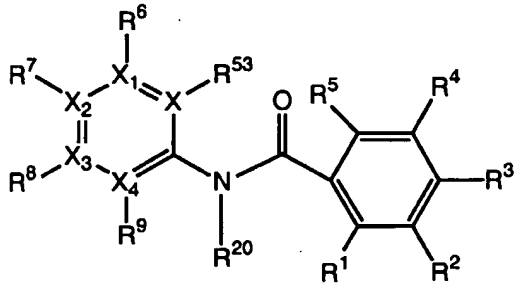
EXPERIMENTAL

Novel compounds of the present invention can be prepared by the synthetic schemes outlined below:

SCHEME-I



Formula A Formula B



Formula I

STEP-1

A mixture of a compound of Formula A (1 eq.), a compound of Formula B (1.2 eq.) and dimethyl acetamide (DMA) is stirred at ambient temperature from about 30 minutes to about 2 hours, or until a TLC analysis indicates absence of the compound of Formula A. The reaction mixture then is diluted with ether or water leading to the formation of a precipitate of a compound of Formula I. This precipitate is isolated and dried. Structural confirmation and compound identification is accomplished by techniques such as proton NMR (^{1}H NMR), mass spectral analysis (MS) and elemental analysis.

Formula I (R^1 = OH)

Conversion of Formula I compounds, where R^1 is O-acetyl, to Formula I compounds, where R^1 is OH, is accomplished by treating a compound of Formula I with a base, preferably aqueous ammonium hydroxide. The reaction mixture is initially clear but formation of a yellowish precipitate indicates the conversion of an O-acetyl group to a hydroxy group. This conversion is generally quantitative. The precipitate is isolated and dried to yield the corresponding compound of Formula I, where R^1 is OH.

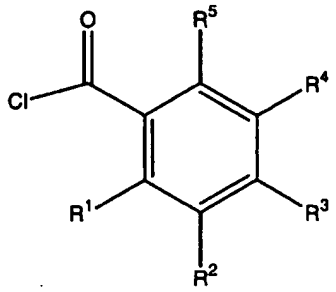
Acid Salts

Acid salts of compound of Formula I can be formed by stirring a compound of Formula I, having at least one amino center, with an acid, preferably a mineral acid such as HCl. This affords the corresponding acid salt of a compound of Formula I as a solid. The solid is isolated and dried. Structural identification is accomplished using techniques such as (^{1}H NMR), MS and elemental analysis.

Synthesis of Starting Materials

Some of the compounds of Formula A and Formula B can be purchased from commercial sources such as Aldrich Chemicals and Lancaster. Compounds of Formula A and Formula B can also be prepared by synthetic methods known to one skilled in the art. Thus compounds of Formula B can be synthesized as described below.

Synthesis of Compounds of Formula B:

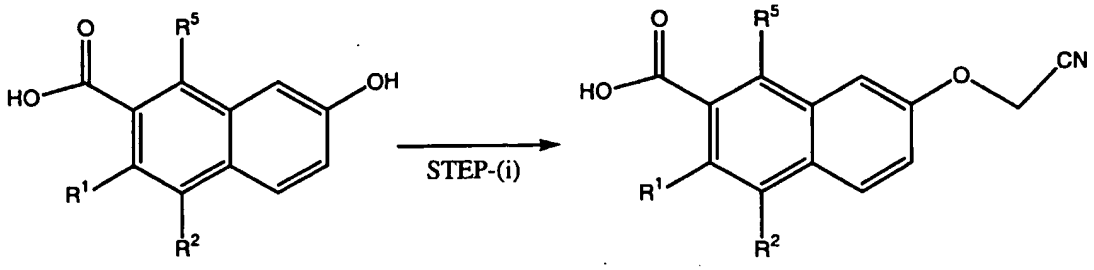


Formula B

Compounds of Formula B are acid chlorides which can be synthesized by dissolving an appropriate carboxylic acid in an appropriate solvent, for example ethyl acetate (EtOAc) with a catalytic amount of DMF, and treating this mixture with about 1.5 equivalents of oxalyl chloride. The resulting reaction mixture is stirred at ambient temperature for about 30 minutes. The solvent is evaporated to obtain a compound of Formula B. These compounds of Formula B can be used without further purification.

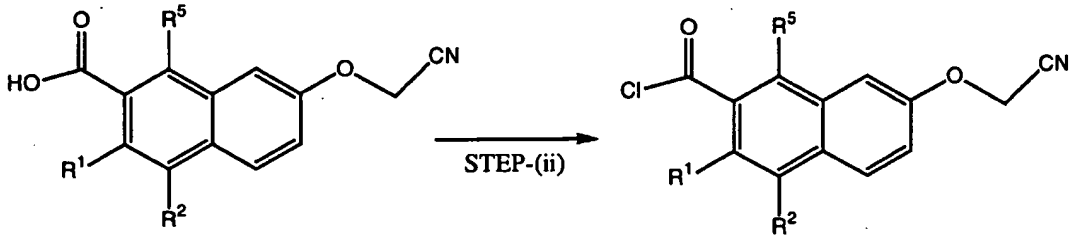
The acetylated carboxylic acid used above can, in turn, be prepared by acetylating the corresponding hydroxy carboxylic acid, e.g., salicylic acid. The procedure comprises combining a suspension of the hydroxy carboxylic acid in acetic anhydride with catalytic amount of acid, e.g., sulfuric acid and agitating this mixture from about 1 to about 3 hours at ambient temperature. The acetylated carboxylic acid falls out of the solution as a solid. This acetylated carboxylic acid then is used as described above.

Scheme II



Formula X

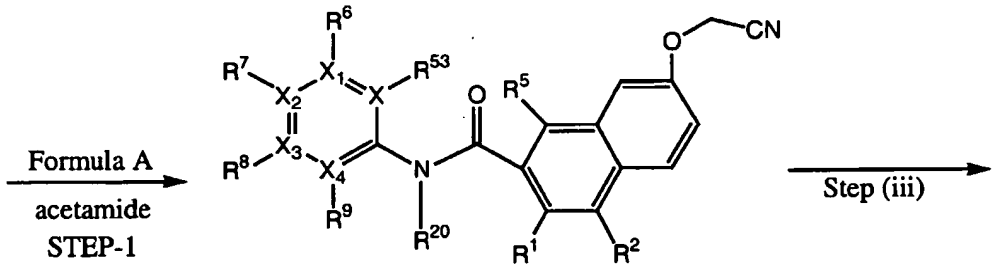
Formula Y



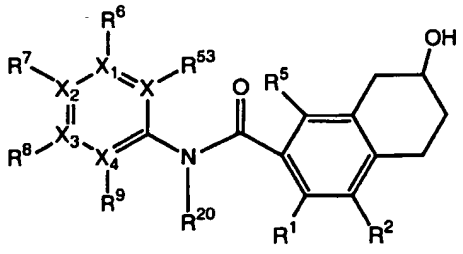
Formula Y

Formula B

5



Formula I



Formula I

STEP-(i)

A compound of Formula X (500 mg, 2.5 mmol) was mixed with DMF (5 ml) and 60% sodiumhydroxide (0.32 g) to form a mixture. The mixture then was stirred for about 30 minutes. The stirred mixture was combined with chloroacetonitrile (0.17 ml, 1.1 eq.) and the new reaction mixture was stirred for about 1 hour followed by dilution with 1N HCl to form a precipitate. The precipitate was isolated and dried to yield a compound of Formula Y.

STEP-(ii)

Compounds of Formula B are acid chlorides which can be synthesized by dissolving an appropriate corresponding carboxylic acid in an appropriate solvent, for example ethyl acetate (EtOAc) with a catalytic amount of DMF, and treating this mixture with about 1.5 equivalents of oxalyl chloride. The resulting reaction mixture is stirred at ambient temperature for about 30 minutes. The solvent is evaporated to obtain a compound of Formula B. These compounds of Formula B can be used without further purification.

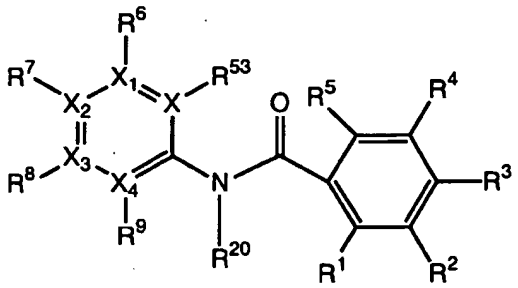
STEP-1

A mixture of a compound of Formula A (1 eq.), a compound of Formula B (1.2 eq.) and dimethyl acetamide (DMA) was stirred at ambient temperature from about 30 minutes to about 2 hours, or until a TLC analysis indicates absence of the compound of Formula A. The reaction mixture then was diluted with ether or water leading to the formation of a precipitate of a compound of Formula I. This precipitate was isolated and dried. Structural confirmation and compound identification was accomplished by techniques such as proton NMR (^{1}H NMR), mass spectral analysis (MS) and elemental analysis.

STEP-(iii)

A compound of Formula I (Ex. 168) was combined with a mixture of methanol and 1N HCl followed. The resulting mixture was further combined with Platinum oxide and this mixture was agitated under hydrogen at 35 PSI for about 1 hour. The agitated mixture was filtered and concentrated to yield an oily substance. The oily substance was purified by preparative HPLC eluting with a gradient of 10-90% solvent A in solvent B (The solvent A was 20 mm HCl, solvent B was acetonitrile) to yield a compound of Formula I (Ex. 175).

Compounds of Formula I wherein $R^2 = SO_3H$



Formula I

A compound of Formula I (R^2 = H) (100 mg, 0.31 mmol) was dissolved in concentrated sulfuric acid (2 ml) and then mixed with a sulfur trioxide-N,N-dimethylformamide complex (120 mg, 0.78 mmol). The resulting solution was heated at about 50 °C for about 10 minutes, and then diluted with water to yield a precipitate. The precipitate was isolated and dried to yield a compound of Formula I wherein $R^2 = SO_3H$ (Ex.173) .

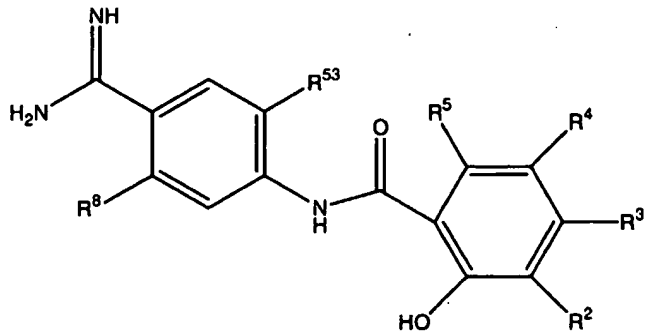
Synthesis of Compounds wherein R^2 = OH or NH_2.

A compound of Formula I (R^2 = H) (120 mg, 0.37 mmol) was suspended in water (6 ml) and the suspension was treated with fuming nitric acid (0.5 mL). The resulting mixture was stirred from about 8 to about 16 hours and the solids were isolated by filtration. The solids then were dissolved in a mixture of methanol (10 mL) and 1N HCl (1 mL), the solution was combined with Palladium(II)hydroxide catalyst (20%) and the resulting reaction mixture was agitated in an atmosphere of hydrogen for about 12 hours. The agitated reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure to yield a residue. The residue was purified and the two components of the residue were separated using reverse phase HPLC to yield two compounds of Formula I wherein R^2 = OH and NH_2 respectively.

Examples

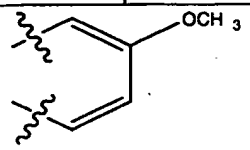
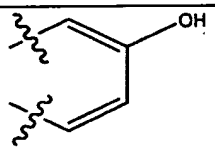
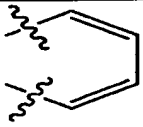
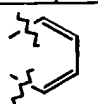
Listed in TABLES-I, II and III are compounds which were synthesized using the procedures discussed above.

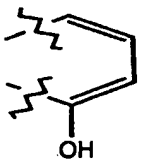
TABLE-I



R^8 and R^{53} represent H, unless noted otherwise.

Ex.	R^2	R^3	R^4	R^5	
1	I	H	CH_3	H	
2	Br	OH	Br	H	
3	I	OH	Br	H	
4	I	NH_2	I	H	
5	Br	H	CH_3	H	
6	Br	NH_2	Br	H	
7	I	H	CH_3	H	$R^8 = F$
8	I	H	F	H	

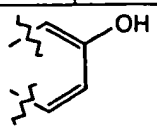
Ex.	R^2	R^3	R^4	R^5	
9	Br	OH	H	H	
10	H			H	
11	H			H	
12	I	H	Cl	H	
13	H			H	
14	Br	H	F	H	
15	Cl	H	H	H	
16	H	OC_2H_5	H	H	
17	Br	OCH_3	Br	H	
18	H	NH_2	H	H	
19	H	CH_3	H	H	
20			H	H	
21	Br	H	Br	H	

Ex.	R^2	R^3	R^4	R^5	
22	H	$OCH_2CH_2OC(O)CH_3$	H	H	
23	Br	CH_3	Br	H	
24	$CH(CH_3)_2$	H	$CH(CH_3)_2$	H	
25	OH	H	H	H	
26	H	H	H	OH	
27	CH_3	H	H	H	
28	Cl	H	Cl	H	
29	Br	H	benzo[1,3]dioxol-5-yl	H	
30	NO_2	H	$NHC(O)CF_3$	H	
31	$CH(CH_3)_2$	H	H	H	
32	H			H	
33	H			H	$R^8 = F$
34	H	OCH_3	H	H	
35	H	$NHC(O)CH_3$	H	H	

Ex.	R^2	R^3	R^4	R^5	
36	H	H	NH_2	H	
37	H	H	CH_3	H	
38	H	H	H	H	
39	H	$OCH_2C(O)NH_2$	H	H	
40	H	H	OCH_3	H	
41	H	OH	H	H	
42	H	$NHC(O)CF_3$	H	H	
43	H	OH	H	OH	
44	H	N=CH-$N(CH_3)_2$	H	H	
45	H	H	I	H	
46	H	(3-phenyl-propylcarbamoyl)-methoxy	H	H	
47	Br	H	3-nitro-phenyl	H	
48	H	H	4-carbamimidoyl-phenylazo	H	

Ex.	R^2	R^3	R^4	R^5	
49	H	OH	Br	H	
50	H	phenethylcarbamoyl-methoxy	H	H	
51	H	H	$NHC(O)CH_3$	H	
52	H	benzylcarbamoyl-methoxy	H	H	
53	H	Cl	H	H	
54	H	H	(3-phenyl-propylamino)-methyl	H	
55	H	H	F	H	
56	H	H	2,4-difluorophenyl-1-yl	H	
57	H	H	3-(4-carbamimidoyl-phenylcarbamoyl)-4-hydroxy-phenylsulfanyl	H	
58	H	(2-morpholin-4-yl-ethyl-carbamoyl)-methoxy	H	H	
59	H	H	Cl	H	
60	H	H	Br	H	
61	H	H	benzo[1,3]dioxol-5-yl	H	

Ex.	R^2	R^3	R^4	R^5	
62	H	[(tetrahydro-furan-2-ylmethyl)-carbamoyl]-methoxy	H	H	
63			OH	H	
64	H	H	2-carboxy-4-mercaptoyl-phenol	H	
65	Ph	H	H	H	
66	H	H	H	H	$R^{53} = CH_3$
67	H	H	1,3-dioxo-1,3-dihydro-isoindol-2-yl	H	
68	H	H	$NHC(O)CF_3$	H	
69	H	H	toluene-4-sulfonylamino	H	
70	H	H	3-nitrophen-1-yl	H	
71	I	H	CH_3	H	$R^8 = F$
72	H	$O(CH_2)_5COOC_2H_5$	H	H	
73	H	$O(CH_2)_5COOH$	H	H	
74	NH_2	H	H	H	

Ex.	R^2	R^3	R^4	R^5	
75	H			H	
76	4-cyano-benzoylamino	H	H	H	
77	NHC(O)-Ph	H	H	H	
78	H	OCH_2Ph	H	H	
79	H	4-ethoxy-carbonyl-cyclohexyloxy	H	H	
80	I	H	CH_3	H	R^{53} = Cl
81	H	4-Carbamimi-doyl-phenyl carbamoyl	OH	H	

Listed below is the proton NMR (^{1}H NMR) and Mass spectral data for compounds listed in TABLE-I.

Ex.1.

^{1}H NMR (DMSO-d_6) δ : 12.42 (s, 1H), 10.91 (s, 1H), 9.33 (s, 2H), 9.02 (s, 2H), 7.98-7.85 (m, 6H), 2.30 (s, 3H)

Mass Spec (M+1) = 396

Ex. 2

^{1}H NMR (DMSO-d_6) δ: 10.8(br, 2H), 9.3(br s, 2H), 8.9(br s, 2H), 8.4(s, 1H), 7.85(m, 4H)

Mass Spec (M+1) = 429.6

Ex. 4

^{1}H NMR (DMSO-d_6) δ: 9.28 (s, 2H), 8.94 (s, 2H), 8.50 (s, 1H), 7.92 (d, 2H, J = 8.91), 7.85 (d, 2H. J = 8.91), 5.90 (s, 2H).

Ex. 5

^{1}H NMR (DMSO-d_6) δ 12.11 (s, 1H), 10.92 (s, 1H), 9.31 (s, 2H), 9.03 (s, 2H), 7.97-7.85 (m, 5H), 7.66 (s, 1H), 2.30 (s, 3H).

Mass Spec (M+1) = 347.7

Ex. 6

^{1}H NMR (DMSO-d_6) δ 10.60 (s, 1H), 9.28 (s, 2H), 8.97 (s, 2H), 8.38 (s, 1H), 7.93 (d, 2H, J = 8.91), 7.85 (d, 2H. J = 8.66).

Ex. 8

^{1}H NMR (DMSO-d_6) δ 9.31 (s, 2H), 8.98 (s, 2H), 8.07 (d, 1H, J = 9.65), 7.99-7.91 (m, 3H), 7.85 (d, 2H, J = 8.66).

Mass Spec (M+1) = 399.7

Ex. 10

^{1}H NMR (DMSO-d_6) δ: 9.2(br), 8.25(s, 1H), 7.85(br s, 4H), 7.31(d, 1H, J=9 Hz), 7.06(d, 1H, J=2.2 Hz), 6.87(dd, 1H, J=2.5, 8.8 Hz), 6.74(s, 1H), 3.78(s, 3H)

Mass Spec (M+1) = 336.6

Ex. 11

^{1}H NMR (DMSO-d_6) δ: 8.28(m, 1H), 8.16(m, 1H), 7.90(m, 2H), 7.83(m, 2H), 7.5(m, 2H), 6.96(m, 2H), 6.74(s, 1H)

Mass Spec (M+1) = 321.9

Ex. 12

^{1}H-NMR (DMSO-d_6) δ: 11.87 (s, 1H), 10.42 (s, 1H), 9.81 (s, 1H), 7.89 (d, 1H, J = 7.97 Hz), 7.78 (d, 2 H, J = 8.09 Hz), 7.43 (s, 3 H), 7.22 (d, 2H, J = 8.56 Hz), 6.80-6.70 (m, 2H), 2.28 (s, 3H).

Mass Spec (M+1) = 284.9

Ex. 13

^{1}H NMR (DMSO-d_6) δ: 11.2(br s, 1H), 10.95(br s, 1H), 9.3(br s, 2H), 9.0(br s, 2H), 8.45(s, 1H), 8.05-7.9(m, 5H), 7.8(d, 1H), 7.55(t, 1H), 7.35(m, 2H)

Mass Spec (M+1) = 306.3

Ex. 14

^{1}H NMR (DMSO-d_6) δ 10.96 S, 1H), 9.31 (s, 2H), 8.98 (s. 2H), 8.03 (d, 1H, J = 8.66), 7.95 (d, 2H, J = 8.42), 7.88-7.85 (m, 3H).

Mass Spec (M+1) = 353.6

Ex. 15

^{1}H NMR (DMSO-d_6) δ 7.89 (d, 2H, J = 8.91 Hz), 7.79 (d, 2H, J = 8.91 Hz), 7.65 (dd, 1H, J = 1.98, 7.92 Hz), 7.19 (dd, 1H, J = 1.98, 7.43 Hz), 6.11 (t, 1H, J = 7.67 Hz).

Mass Spec (M+1) = 289.7

Ex. 16

^{1}H NMR (DMSO-d_6) δ 12.08 (s, 1H), 10.56 (s, 1H), 9.27 (s, 2H), 8.95 (s, 2H), 7.99 (d, 2H, J = 8.97 Hz), 7.94 (s, 1H), 7.85 (d, 2H, J = 8.97 Hz), 6.59-6.53 (m, 2H), 4.07 (q, 2H, J = 6.86 Hz), 1.43 (t, 3H, J = 6.86 Hz).

Mass Spec (M+1) = 299.9

Ex. 17

^{1}H NMR (DMSO-d_6) δ 10.30 (s, 1H), 9.23 (s, 2H), 8.91 (s, 2H), 7.92 (d, 2H, J = 8.42 Hz), 7.83-7.74 (m, 3H), 6.19 (d, 1H, J = 8.91 Hz), 6.10 (s, 1H).

Mass Spec (M+1) = 270.7

Ex. 18

^{1}H NMR (DMSO-d_6) δ 9.31 (s, 2H), 8.98 (s, 2H), 8.47 (s, 1H), 7.95-7.86 (m, 4H), 3.86 (s, 3H).

Mass Spec (M+1) = 443.8

Ex. 19

^{1}H NMR (DMSO-d_6) δ :11.7(br s, 1H), 10.65(br s, 1H), 9.4(br s, 2H), 9.05(br s, 2H), 7.9(m, 5H), 6.8(m, 2H), 2.3(s, 3H).

Ex. 23

^{1}H NMR (DMSO-d_6) δ: 12.8(br s, 1H), 11.05(s, 1H), 9.3(br s, 2H), 9.08(br s, 2H), 8.4(d, 1H, J=2.2 Hz), 7.89(m, 4H), 3.55(s, 3H)

Mass Spec (M+1) = 427.6

Ex. 30

^{1}H NMR (DMSO-d_6) δ: 11.6(br s, 1H), 11.5(br s, 1H), 9.3(br s, 2H), 8.9(br s, 2H), 8.5(s, 1H), 8.3(s, 1H), 7.9(m, 4H)

Mass Spec (M+1) = 411.8

Ex. 32

^{1}H NMR (DMSO-d_6) δ: 9.2(br, 4H), 8.26(d, 1H, J=3.3 Hz), 7.87(br s, 4H), 7.11(d, 1H, J=3.2 Hz), 7.02(d, 1H, J=3 Hz), 6.73(m, 1H), 6.60(m, 1H)

Mass Spec (M+1) = 321.9

Ex. 36

^{1}H NMR (DMSO-d_6) δ: 10.75(s, 1H), 10.25(br , 3H), 9.35(br s, 2H), 9.05(br s, 2H), 7.95(m, 4H), 7.85(d, 1H), 7.45(d, 1H), 7.2(d, 1H)

Mass Spec (M+1) = 270.8

Ex. 38

N-(4-carbamimidoyl-phenyl)-2-hydroxy-benzamide

A solution of 4-aminobenzonitrile (1 g; 7.57 mmol) in THF (25 mL) was combined with acetylsalicyloyl chloride (11.5 g; 1 eq.) and Et_3N (2 mL). This mixture

was agitated for 8-12 hours and then diluted with ethyl acetate (50 mL). The diluted mixture was washed in succession with 1M HCl solution (15 mL), brine (50 mL), dried ($MgSO_4$) and concentrated under reduced pressure to yield a yellow colored oily residue . Purification of the oily residue by flash chromatography yielded 4-(2-acetoxybenzamido)-benzonitrile (0.9g).

The above 4-(2-acetoxybenzamido)-benzonitrile (0.9 g) was dissolved in a 1:3 mixture of dioxane:ethyl acetate (15 mL) and the resulting mixture was cooled to a temperature of from about 0°C to about 15°C. The cold reaction mixture was saturated with gaseous HCl, the reaction vessel was sealed and the reaction mixture was agitated from about 8 to about 12 hours. The reaction mixture was concentrated under reduced pressure to yield a solid. This solid was dissolved in a 2M ammonia solution in ethanol and the resulting mixture was agitated in a sealed reaction vessel from about 8 to about 16 hours. The reaction mixture was concentrated under reduced pressure to yield an oily residue. The oily residue was purified using purification techniques known to one skilled in the art, for example HPLC, to yield N-(4-Carbamimidoyl-phenyl)-2-hydroxy-benzamide (27 mg).

^{1}H NMR (DMSO-d_6) δ: 11.58(br. S, 1H), 10.75(br S, 1H), 9.26(br S, 2H), 8.94(br S, 2H), 7.93(dd, 2H, J=8.8, 1.8 Hz), 7.89(dd, 1H, J=6,1.4 Hz), 7.82(dd, 2H, J=9, 2.1 Hz), 7.41(m, 1H), 7.01(d, 1H, J=8 Hz), 6.95(m, 1H).

Mass Spec (M+1) = 255.9

Ex. 39

^{1}H NMR (DMSO-d_6) δ: 12.1(s, 1H), 10.6(s, 1H), 9.3(br s, 2H), 9.1(br s, 2H), 8.0(m, 3H), 7.85(m, 2H), 7.65(br s, 1H), 7.4(br s, 1H), 6.6(m, 2H).

Mass Spec (M+1) = 329.3

Ex. 41

^{1}H NMR (DMSO-d_6) δ: 11.95(br s, 1H), 10.5(br s, 1H), 10.35(br s, 1H), 9.25(br s, 2H), 8.9(br s, 2H), 7.9(m, 5H), 6.4(m, 2H).

Mass Spec (M+1) = 271.7

Ex. 45

^{1}H NMR (DMSO-d_6) δ: 11.65(br, 1H), 10.7(s, 1H), 9.3(br s, 2H), 9.0(br s, 2H), 8.15(s, 1H), 7.95(d, 2H), 7.85(d, 2H), 7.7(d, 1H), 6.9(d, 1H)

Mass Spec (M+1) = 382.1

Ex. 49

^{1}H NMR (DMSO-d_6) δ: 12.05(br s, 1H), 11.3(br s, 1H), 10.5(s, 1H), 9.3(br s, 2H), 9.0(br s, 2H), 8.2(s, 1H), 7.9(m, 4H), 6.7(s, 1H).

Ex. 63

^{1}H NMR (DMSO-d_6) δ: 9.0(br, 4H), 8.28(d, 1H), 7.89(m, 2H), 7.78(m, 3H), 7.33(m, 1H), 7.18(m, 1H).

Mass Spec (M+1) = 322.3

Ex. 65

^{1}H NMR (DMSO-d_6) δ: 12.45(s, 1H), 10.9(s, 1H), 9.3(s, 2H), 8.95(s, 2H), 8.1(d, 1H), 7.95(d, 2H), 7.55(m, 3H), 7.4(m, 3H), 7.1(t, 1H).

Mass Spec (M+1) = 331.9

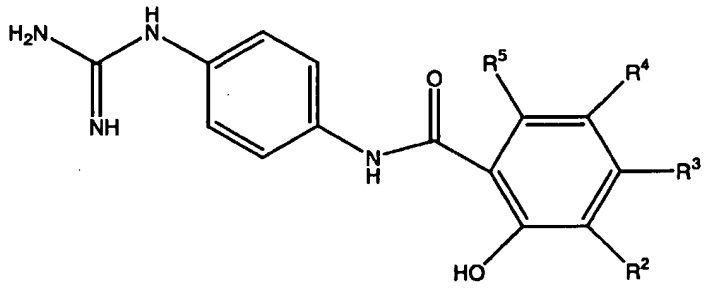
Ex. 68

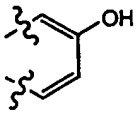
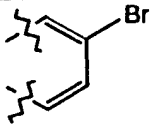
^{1}H NMR (DMSO-d_6) δ: 11.5(br s, 1H), 11.25(s, 1H), 10.7(s, 1H), 9.3(br s, 2H), 8.9(br s, 2H), 8.15(d, 1H), 8.0(d, 2H), 7.9(d, 2H), 7.65(d, 1H), 7.1(d, 1H).

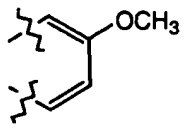
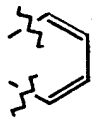
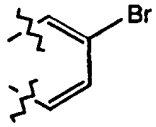
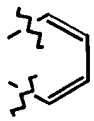
Mass Spec (M+1) = 366.8

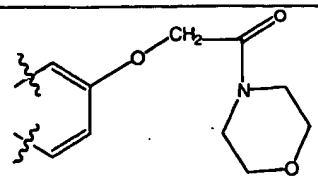
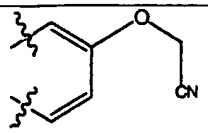
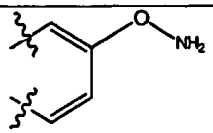
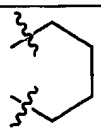
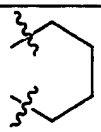
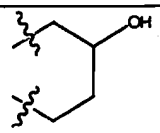
Listed in TABLE-II below are compounds wherein R^7 is a guanidinyl group ($NH\text{-}C(=NH)NH_2$).

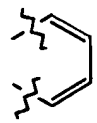
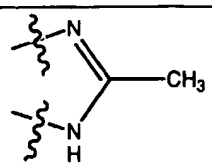
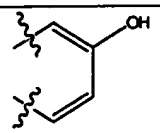
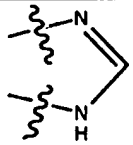
TABLE-II



Ex.	R^2	R^3	R^4	R^5	
150	H			H	
151	H			H	
152	Br			H	
153	Br			H	

Ex.	R^2	R^3	R^4	R^5	
154	H			H	
155	Cl			H	
156	H			H	
157	I			H	
158	H	Ph	H	H	
159	H			H	
160	H	CH_3	H	H	
161	H			H	$R^6 = F$
162	Br	H	CH_3	H	
163	I	H	CH_3	H	
164	H	OC_2H_5	H	H	
165	I	OH	Br	H	

Ex.	R^2	R^3	R^4	R^5	
166	Br	H	Br	H	
167	H		H	H	
168	H		H	H	
169	H		H	H	
170	NH_2		H	H	
171	OH		H	H	
172	H		H	H	

Ex.	R^2	R^3	R^4	R^5	
173	SO_3H		H	H	
174	H		H	H	
175	H		H	H	
176	H		H	H	

Listed below is the proton NMR (^{1}H NMR) and Mass spectral data for compounds listed in TABLE-II.

EX. 150

3-Acetoxy-2-naphthoic acid:

A mixture of 3-hydroxy-2-naphthoic acid (1 g, 5.3 mmol) and acetic anhydride (1 mL) was combined with con. sulfuric acid (2 drops) resulting in a solidified mixture in about 30 minutes. The solid was washed with acetic acid (15 mL) and recrystallized using a 1:1 mixture of methanol:water to yield 3-Acetoxy-2-naphthoic acid (0.68 g; 56% yield) in the form of yellow needles.

^{1}H-NMR (DMSO-d_6) δ: 8.60 (s, 1H), 8.11 (d, 1H, J = 8.1 Hz), 7.95 (d, 1H, J = 8.1 Hz), 7.71 (s, 1H), 7.66 (t, 1H, J = 7.0 Hz), 7.58 (t, 1H, 7.5 Hz), 2.30 (s, 3H).

N-(3-hydroxy-2-naphthoyl)-4-aminophenyl guanidine hydrochloride:

A suspension/mixture of 3-acetoxy-2-naphthoic acid (2.0 g, 8.7 mmol), ethyl acetate (17 mL) and catalytic amount of DMF (0.2 mL) was combined with oxalyl chloride (1.1 mL, 13 mmol) to form a mixture. The mixture was agitated for an hour. The agitated mixture was concentrated under reduced pressure to yield 3-acetoxy-2-naphthoyl chloride as a yellowish solid. The preceding naphthoyl chloride and 4-aminophenylguanidine hydrochloride (1.94 g, 8.7 mmol) was suspended in N,N-dimethyl acetamide (DMA). This suspension was agitated for about 8 to 16 hours to form a solution. The solution was diluted with ether (150 mL) and the diluted reaction mixture was agitated vigorously for about 5 minutes forming a precipitate. The precipitate was isolated and dried to yield N-(3-acetoxy-2-naphthoyl)-4-aminophenyl guanidine.

An aqueous mixture of the preceding N-(3-acetoxy-2-naphthoyl)-4-aminophenyl guanidine hydrochloride was treated with 2N NaOH (18 mL, 36 mmol) at a temperature of about 70^0C for about 8 hours. Conversion of the acetoxy group to a hydroxy group was confirmed by MS (CI) analysis. The reaction mixture then was acidified with 6M HCl leading to the formation of a golden-yellow colored precipitate. This precipitate was isolated, washed with water and dried to yield N-(3-hydroxy-2-naphthoyl)-4-aminophenyl guanidine hydrochloride (2.75 g). This guanidine hydrochloride was purified by flash chromatography.

The purified N-(3-hydroxy-2-naphthoyl)-4-aminophenyl guanidine hydrochloride was dissolved in aqueous dilute NaOH. This NaOH solution was acidified to a pH of about 6-7 using 6 M HCl leading to precipitate formation. The precipitate was isolated and dried to yield N-(3-hydroxy-2-naphthoyl)-4-aminophenyl guanidine hydrochloride as a tan colored solid (1.36 g; 44% yield).

^{1}H NMR (DMSO-d_6) δ : 11.33 (3, 1H), 10.71 (s, 1H), 9.84 (s, 1H), 8.49 (s, 1H), 7.92 (d, 1H, J = 8.2 Hz), 7.8 (s, 1H, J = 8.5 Hz), 7.75 (d, 1H, J = 8.3 Hz), 7.55-7.30 (m, 7H), 7.25 (d, 2H, J = 8.6 Hz).

Mass Spec (M+1) = 321.0

Ex. 152

^{1}H-NMR (DMSO-d_6) δ: 11.1 (s, 1H), 9.8 (s, 1H), 8.7 (s, 1H), 8.1 (d, 1H), 8.0 (d, 1H), 7.8 (d, 2H), 7.7 (t, 1H), 7.5-7.3 (m, 4H), 7.2 (d, 2H).

Mass Spec (M+1) = 400.7

Ex. 155

^{1}H-NMR (DMSO-d_6) δ: 11.9 (br s, 1H), 10.9 (s, 1H), 9.7 (s, 1H), 8.6 (s, 1H), 8.0 (d, 1H), 7.9 (d, 1H), 7.7 (d, 2H), 7.6 (t, 1H), 7.5-7.3 (m, 4 H), 7.2 (d, 2H).

Mass Spec (M+1) = 354.8

Ex. 157

^{1}H-NMR (DMSO-d_6) δ: 12.73 (s, 1H), 11.15 (s, 1H), 9.93 (s, 1H), 8.90 (s, 1H), 8.00 (d, 1H, J = 8.55 Hz), 7.95 (d, 1H, J = 8.07 Hz), 7.86 (d, 2H, J = 8.71 Hz), 7.70 (t, 1H, J = 7.66 Hz), 7.60-7.45 (m, 4H), 7.29 (d, 2H, J = 8.65 Hz).

Mass Spec (M+1) = 446.9

Ex. 159

^{1}H-NMR (DMSO-d_6) δ: 11.30 (s, 1H), 10.75 (s, 1H), 10.00 (s, 1H), 8.47 (s, 1H), 7.93 (d, 1H, J = 8.18 Hz), 7.79 (s, 1H), 7.77 (d, 1H, J = 8.52 Hz), 7.65 (d, 1H, J = 8.52 Hz), 7.58-7.32 (m, 7H), 7.01 (d, 1H, J = 8.18 Hz).

Mass Spec (M+1) = 320.9

Ex. 160

^{1}H-NMR (DMSO-d_6) δ: 11.87 (s, 1H), 10.42 (s, 1H), 9.81 (s, 1H), 7.89 (d, 1H, J = 7.97 Hz), 7.78 (d, 2 H, J = 8.09 Hz), 7.43 (s, 3 H), 7.22 (d, 2H, J = 8.56 Hz), 6.80-6.70 (m, 2H), 2.28 (s, 3H). Mass Spec (M+1) = 284.9.

Ex. 162

^{1}H NMR (DMSO-d_6) δ: 12.60 (s, 1H), 10.73 (s, 1H), 9.94 (s, 1H), 7.79-7.94 (m, 2H), 7.79 (d, 2H, 8.91), 7.65 (s, 1H), 7.50 (s, 2H), 7.27 (d, 2H, J = 8.66), 2.30 (s, 3H).

Mass Spec (M+1) = 364.8

Ex. 163

^{1}H NMR (DMSO-d_6) δ: 12.83 (s, 1H), 10.71 (s, 1H), 9.89 (s, 1H), 7.98 (s, 1H), 7.84 (s, 1H), 7.78 (d, 2H, J = 8.91), 7.48 (s, 2H), 7.27 (d, 2H, J = 8.91), 2.29 (s, 3H).

Mass Spec (M+1) = 410.8

Ex. 164

^{1}H NMR (DMSO-d_6) δ: 12.33 (s, 1H), 10.33 (s, 1H), 9.75 (s, 1H), 7.99 (d, 1H, J = 8.71), 7.78 (d, 2H, J = 8.71), 7.40 (s, 2H), 7.23 (d, 2H, J = 8.71), 6.55 (dd, 1H, J = 8.71, 2.38), 6.49 (d, 1H, J = 2.38), 4.07 (q, 2H, J = 6.86 Hz), 1.43 (t, 3H, J = 6.86 Hz).

Mass Spec (M+1) = 314.8.

Ex. 167

^{1}H-NMR (d_6-DMSO) δ (ppm): 11.07 (s, 1H), 11.01 (br s, 1H), 10.73 (s, 1H), 8.73 (s, 1H), 8.32 (s, 1H), 8.23-8.08 (m, 4 H), 7.69 (d, 1H, J = 8.8 Hz), 7.29 (s, 2H), 7.20 (d, 1H, J = 8.8 Hz), 7.10 (d, 1H, J = 8.8 Hz), 4.90 (s, 2H), 3.60-3.44 (m, 8 H).

MS (ES) calc. 464.5, found 465.2 (MH+).

This compound was prepared by the following process:

3,7-Dihydroxy-naphthalene-2-carboxylic acid benzyl ester

A mixture of 3,7-dihydroxy-naphthalene-2-carboxylic acid (10.0 g, 49 mmol) and $NaHCO_3$ (10.3 g, 123 mmol) in 70 mL of N,N-dimethylformamide was agitated for approximately 12 hours at ambient temperature and at about 70°C for an additional 4 hours. The mixture was cooled to about 40°C and then combined with benzyl bromide (7 mL, 59 mmol). The resulting mixture was agitated at about 70°C for about 12 hours. The preceding agitated reaction mixture was concentrated under reduced

pressure, diluted with AcOEt and the diluted mixture was sequentially washed with satd. $NaHCO_3$, satd NaCl, 0.5 M HCl, and satd. NaCl, dried (Na_2SO_4) and concentrated under reduced pressure to afford a brown oil. The brown oil was diluted with hexanes to form a precipitate which was isolated to afford the benzyl ester as a golden powder (11.65 g, 81%). ^{1}H-NMR (d_6-DMSO) δ (ppm): 9.95 (s, 1H), 9.62 (s, 1H), 8.23 (s, 1H), 7.60 (d, 1H, J = 8.8 Hz), 7.50 (d, 2H, J = 7.3 Hz), 7.43-7.34 (m, 3 H), 7.22 (s, 1H), 7.13-7.09 (m, 2H), 5.40 (s, 2H).

3-Hydroxy-7-(2-morpholin-4-yl-2-oxo-ethoxy)-naphthalene-2-carboxylic acid benzyl ester A mixture of morpholine (2.16 mL, 25 mmol) and anhydrous ether (30 mL) was cooled (-10°C) and treated drop wise with a solution of bromoacetyl bromide (5.0 g, 25 mmol) in ether (20 mL). Triethyl amine (3.5 mL, 25 mmol) then was added drop wise to the reaction mixture to form a cream colored reaction mixture. The creamy reaction mixture was agitated at about 20°C for about 6 hours. The reaction solids were isolated and rinsed with ether. The combined ether fractions were concentrated under reduced pressure to afford N-(2-bromoacetyl)-morpholine (3.37 g) as a reddish oil, which was used without further purification.

A solution of the N-(2-bromoacetyl)-morpholine (2.09 g, 10 mmol) in acetone (5 mL) was introduced in a drop wise manner into a mixture of 3,7-Dihydroxy-naphthalene-2-carboxylic acid benzyl ester (2.69 g, 9.1 mmol) and K_2CO_3 (1.39 g, 10.1 mmol) in 15 mL of acetone. The combined mixture was heated to reflux for about 12 hours, at which time another 0.2 g (1.0 mmol) of N-(2-bromoacetyl)-morpholine and 0.24 g of K2CO3 (1.7 mmol) were added and the heating continued for an additional 3 hours. The mixture was cooled to ambient temperature, diluted with AcOEt, washed with

water and satd. NaCl, dried (Na2SO4) and concentrated under reduced pressure to yield an oily residue. The oily residue was purified by chromatography (silica) using a gradient elution employing 50 to 80% AcOEt in hexanes. The title compound was obtained as a yellow foam (1.06 g, 28%). ^{1}H-NMR (d_6-DMSO) δ (ppm): 10.08 (s, 1H), 8.31 (s, 1H), 7.687 (d, 1H, J = 9.2 Hz), 7.50 (d, 2H, J = 7.0 Hz), 7.44-7.37 (m, 3H), 7.29 (s, 1H), 7.23 (d, 2H, J = 8.8 Hz), 5.41 (s, 2H), 4.85 (s, 2H), 3.59-3.44 (m, 8H).

3-Acetoxy-7-(2-morpholin-4-yl-2-oxo-ethoxy)-naphthalene-2-carboxylic acid chloride

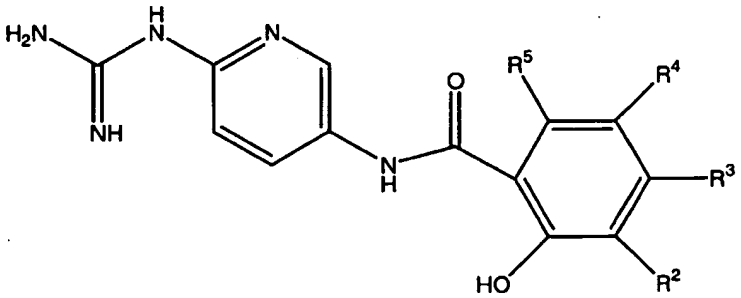
3-Hydroxy-7-(2-morpholin-4-yl-2-oxo-ethoxy)-naphthalene-2-carboxylic acid benzyl ester (1.06 g, 2.5 mmol) was hydrogenated at atmospheric pressure in 10 mL of tetrahydrofuran over 10% Pd-C (wet) for 2 hours. The catalyst was removed by filtration, and solvent was removed under reduced powder to yield the carboxylic acid as a yellow solid (0.77 g, 93%) was used without further purification.

3-Hydroxy-7-(2-morpholin-4-yl-2-oxo-ethoxy)-naphthalene-2-carboxylic acid (from above) was moistened with 3 mL of acetic anhydride and 2 drops of conc. H_2SO_4. The resulting heterogeneous mixture was agitated for 20 min, and the undissolved solids were dissolved by adding 1 mL glacial AcOH. The resulting reaction mixture was concentrated under reduced pressure, the concentrated reaction mixture was diluted with AcOEt (250 mL), dried (Na_2SO_4) and concentrated under reduced pressure to afford the 3-Acetoxy-7-(2-morpholin-4-yl-2-oxo-ethoxy)-naphthalene-2-carboxylic acid as a pale yellow oil, which was taken directly onto the next step.

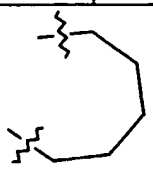
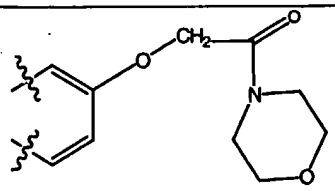
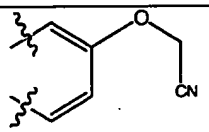
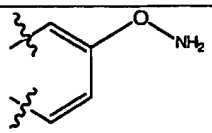
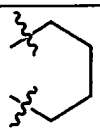
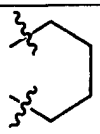
Oxalyl chloride (0.25 mL, 2.8 mmol) was added drop wise to a mixture of the 3-Acetoxy-7-(2-morpholin-4-yl-2-oxo-ethoxy)-naphthalene-2-carboxylic acid (from above), 5 mL of 1,4-dioxane and 0.1 mL of N,N-dimethylformamide. The resulting solution was agitated for about 1 hour. The agitated reaction mixture was concentrated under reduced pressure to yield the acyl chloride which was used without further purification coupling with the appropriate aniline derivative to yield the compound of Example 167.

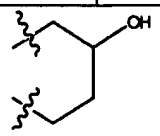
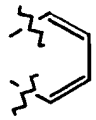
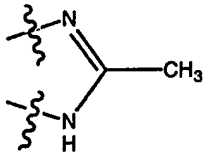
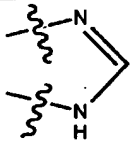
TABLE-III below lists compounds wherein R^7 is a guanidinyl group (NH-C(=NH)NH_2) and X_1 represents a nitrogen atom.

TABLE-III



Ex.	R^2	R^3	R^4	R^5
200	H	H	H	H
201	H			H
202	H	OC_2H_5	H	H
203	H	H	CH_3	H

Ex.	R^2	R^3	R^4	R^5
204	H	H	H	OH
205	I	H	CH_3	H
206	H	CH_3	H	H
207	H			H
208	H			H
209	H			H
210	H			H
211	NH_2			H
212	OH			H

Ex.	R^2	R^3	R^4	R^5
213	H			H
214	SO_3H			H
215	H			H
216	H			H

Listed below is the proton NMR (^{1}H NMR) and Mass spectral data for compounds listed in TABLE-III.

Ex. 200

^{1}H NMR (DMSO-d_6) δ: 11.67 (s, 1H), 11.26 (s, 1H), 10.59 (s, 1H), 8.71 (d, 1H, J = 2.48), 8.21-8.17 (m, 3H), 7.95 (dd, 1H, J = 1.24, 8.17), 7.45 (td, 1H, J = 1.73, 8.91, 8.42), 7.11 (d, 1H, J = 8.91), 7.00 (d, 1H, J = 8.91), 6.96 (d, 1H, J = 7.43).

Mass Spec (M+1) = 271.8

Ex. 201 : 3-hydroxy-naphthalene-2-carboxylic acid (6-guanidino-pyridin-3-yl)-amide

This compound was prepared by reacting 3-acetoxy-naphthalene-2-carboxylic acid chloride (alternatively named as acetic acid 3-chlorocarbonyl-naphthalen-2-yl ester) with N-(5-Amino-pyridin-2-yl)-guanidine hydrochloride. N-(5-Amino-pyridin-2-yl)-guanidine hydrochloride was prepared as described below.

N-(5-Amino-pyridin-2-yl)-guanidine hydrochloride

The first step comprised synthesis of N-(5-nitro-pyridin-2-yl)-guanidine using the procedure of Carbon and Tabata described in *J. Org. Chem* (1962) 2504-7. ^{1}H NMR (DMSO-d_6) δ : 12.23 (s, 1H), 9.12 (d, 1H, J = 2.97 Hz), 8.62 (dd, 1H, J = 2.97, 8.91 Hz), 8.49 (s, 2H), 7.26 (d, 1H, 8.91 Hz).

The second step comprised synthesizing N-(5-amino-pyridin-2-yl)-guanidine hydrochloride by preparing a mixture of N-(5-nitro-pyridin-2-yl)-guanidine hydrochloride (15.82 g; 73 mmol) and 10% Pd/C (100mg) and methanol (1L). This mixture then was agitated in an atmosphere of hydrogen for 2 hours. The agitated

mixture was filtered and the filtrate concentrated to yield N-(5-amino-pyridin-2-yl)-guanidine hydrochloride (13.4 g) as a yellow solid.

^{1}H NMR (DMSO-d_6) δ : 10.88 (s, 1H), 8.01 (s, 2H), 7.65 (d, 1H, J = 2.72 Hz), 7.09 (dd, 1H, J = 2.72, 8.66 Hz), 6.80 (d, 1H, J = 8.66 Hz), 5.29 (d, 2H, J = 4.70 Hz).

5

3-acetoxy-naphthalene-2-carboxylic acid chloride (alternatively named as acetic acid 3-chlorocarbonyl-naphthalen-2-yl ester)

The acid chloride, above, was prepared by treating a mixture of 2-acetoxy-3-naphthoic acid (5 g, 22 mmol), EtOAc (80 ml) and DMF (3 drops) with oxalyl chloride 10 (2.8 ml, 1.5 eq). The resulting reaction mixture was agitated for 0.5 h and the agitated mixture was concentrated *in vacuo* to a yield 3-acetoxy-naphthalene-2-carboxylic acid chloride as a yellow solid.

EX.: 201 3-hydroxy-naphthalene-2-carboxylic acid (6-guanidino-pyridin-3-yl)-amide

15 The above acid chloride (1 eq.) was mixed with DMA (20 ml) and N-(5-amino-pyridin-2-yl)-guanidine hydrochloride (5.33 g, 1.3 eq) and the resulting mixture was agitated for 8-16 hours under an atmosphere of nitrogen. The agitated reaction mixture then was mixed with conc. ammonium hydroxide (150 ml) to form a yellow precipitate. The precipitate was isolated, dried and mixed with 1 M HCl . The 20 mixture was agitated for 2 h, the resulting solids were isolated and dried to yield 3-hydroxy-naphthalene-2-carboxylic acid (6-guanidino-pyridin-3-yl)-amide (6.4 g, 78%) as a pale yellow solid.

^{1}H NMR (DMSO-d_6) δ: 11.24 (s, 1H), 11.19 (s, 1H), 10.77 (s, 1H), 8.77 (d, 1H, J = 2.23), 8.49 (s, 1H), 8.24 (dd, 1H, J = 2.48, 8.91), 8.21 (s, 1H), 7.93 (d, 1H, J = 7.92),

7.77 (d, 1H. J = 8.42), 7.52 (t, J = 6.93, 7.18), 7.39-7.34 (m, 2H), 7.13 (d, 1H, J = 8.91).

Mass Spec (M+1) = 321.8

Ex. 202

^{1}H NMR (DMSO-d_6) δ 8.60 (s, 1H), 8.06 (d, 1H, J = 8.66), 7.73 (d, 1H, J = 8.66), 6.97 (d, 1H, J = 8.91), 6.14-6.10 (m, 2H), 3.95 (q, 2H, J = 6.68), 1.28 (t, 3H, J = 6.68).

Mass Spec (M+1) = 315.8

Ex. 203

^{1}H NMR (DMSO-d_6) δ 8.57 (s, 1H), 8.02 (d, 1H, J = 8.91), 7.65 (s, 1H), 7.07 (d, 1H, J = 8.17), 6.92 (d, 1H J = 8.91), 6.69 (d, 1H, J = 8.42), 2.20 (s, 3H).

Mass Spec (M+1) = 285.8

Ex. 205

^{1}H NMR (DMSO-d_6) δ 11.37 (s, 1H), 10.86 (s, 1H), 8.67 (d, 1H, J = 2.23), 8.23-8.16 (m, 3H), 7.98 (s, 1H), 7.84 (d, 1H, J = 1.73), 7.13 (d, 1H, J = 8.91), 2.29 (s, 3H).

Mass Spec = 411.7

Ex. 206

^{1}H NMR (DMSO-d_6) δ 8.69 (d, 1H, J = 2.72), 8.17 (dd, 1H, J = 2.72, 8.91), 7.87 (d, 1H, J = 7.92), 7.09 (d, 1H, J = 8.66), 6.79 (s, 1H), 6.76 (d, 1H, J = 8.42), 2.29 (s, 3H).

Mass Spec (M+1) = 285.9

UTILITY

Proteases play a significant role in the progression of Cancer. Compounds of the present invention are useful as protease inhibitors. Their inhibitory activity

includes inhibition of urokinase (uPA) which has been postulated to have therapeutic value in treating cancers such as lung cancer, breast cancer, pancreatic cancer, colon cancer, ovarian cancer, bone cancer and the like.

The compounds of the present invention are also useful as anticoagulants for the treatment or prevention of thromboembolic disorders in mammals. The term "thromboembolic disorders" as used herein includes arterial or venous cardiovascular or cerebrovascular thromboembolic disorders, including, for example unstable angina, first or recurrent ischemic attack, stroke, atherosclerosis, venous thrombosis, deep vein thrombosis, thrombophlebitis, arterial embolism, kidney embolisms, and pulmonary embolisms. The anticoagulant effect of compounds of the present invention is believed to be due to the inhibition of Factor Xa (FXa), Factor VIIa (FVIIa), and thrombin.

Some of the compounds of the present invention show selectivity between uPA and FXa, with respect to their inhibitory properties. The effectiveness of compounds of the present invention as inhibitors of Urokinase and Factor Xa is determined by using synthetic substrates and purified Urokinase and purified human Factor Xa respectively.

The rates of hydrolysis by the chromogenic substrates were measured both in the absence and presence of compounds of the present invention. Hydrolysis of the substrates result in the release of a chromogenic moiety, which is monitored spectrophotometrically by measuring the increase in absorbance at 405 nano meter (nm). A decrease in the rate of absorbance change at 405 nm in the presence of a inhibitor is indicative of enzyme inhibition. The results of this assay are expressed as the inhibitory constant, Ki app. Factor Xa determinations were made in

50 mM Tris buffer, pH 7.5, containing 1M NaCl, 5 mM $CaCl_2$, 0.05% Tween-20, and 1.5 mM EDTA. Values of Ki app. were determined by allowing 2-3 nM human Factor Xa (Haematologic Technologies, VT, USA) to react with the substrate (1 mM) in the presence of an inhibitor. Hydrolysis of the chromogenic substrate is followed spectrophotometrically at 405 nm for five minutes. The enzyme assay routinely yielded linear progression curves under these conditions. Initial velocity measurements calculated from the progress curves by a kinetic analysis program (Batch Ki; Peter Kuzmic, BioKin, Ltd., Madison, WI) were used to determine Ki app.

Urokinase inhibition determinations were made in 50 mM Tris (pH 7.5), 150 mM NaCl, 0.05% Tween-20, 0.002% antifoam, and 1 mM EDTA. human Urokinase (from American Diagnostica, CT, USA). Values of Ki app. were determined by allowing 20 nM human Urokinase to react with the Pefachrome substrate (0.3 mM, Centerchem, CT, USA) in the presence of an inhibitor. Hydrolysis of the chromogenic substrate is followed spectrophotometrically at 405 nm for five minutes. The enzyme assay routinely yielded linear progression curves under these conditions. Initial velocity measurements calculated from the progress curves by a kinetic analysis program (Batch Ki; Peter Kuzmic, BioKin, Ltd., Madison, WI) were used to determine Ki app.

Table IV lists inhibition constants (Ki app.) for representative compounds of the present invention. These values are for uPA and FXa.

TABLE-IV

Ex.	uPA Ki μM	FXa Ki μM
1	0.16	0.88
5	0.29	0.84
24	2.9	34
201	0.326	130
205	5.5	290

Definitions

The compounds of the present invention may have asymmetric centers. Compounds of the present invention containing an asymmetrically substituted atom may be isolated in optically active or racemic forms. It is well known in the art how to prepare optically active forms, such as by resolution of materials. Many geometric isomers of olefins, C=N double bonds, and the like can be present in the compounds described herein, and all such stable isomers are contemplated in the present invention. Cis and trans geometric isomers of the compounds of the present invention are described and may be isolated as a mixture of isomers or as separated isomeric forms. All chiral, diastereomeric, racemic forms and all geometric isomeric forms of a structure (representing a compound of Formula I) are intended, unless the specific stereochemistry or isomeric form is specifically indicated.

As used herein, the following terms and abbreviations have the following meaning, unless indicated otherwise.

The term "prodrug" is intended to represent covalently bonded carriers which are capable of releasing the active ingredient of Formula I, when the prodrug is administered to a mammalian subject. Release of the active ingredient occurs *in vivo*. Prodrugs can be prepared by techniques known to one skilled in the art. These techniques generally modify appropriate functional groups in a given compound. These modified functional groups however regenerate original functional groups by routine manipulation or *in vivo*. Prodrugs of compounds of Formula I include compounds wherein a hydroxy, amidino, guanidino, amino, carboxylic or a similar group is modified.

"Pharmaceutically acceptable salts" is as understood by one skilled in the art. Thus a pharmaceutically acceptable salt includes acid or base salts of compounds of Formula I. Illustrative examples of pharmaceutically acceptable salts are mineral acid (hydrochloric acid, hydrobromic acid, phosphoric acid, and the like) salts, organic acid (acetic acid, propionic acid, glutamic acid, citric acid and the like) salts, quaternary ammonium (methyl iodide, ethyl iodide, and the like) salts. It is understood that the pharmaceutically acceptable salts are non-toxic. Additional information on suitable pharmaceutically acceptable salts can be found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, which is incorporated herein by reference.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not. For

example, the phrase "optionally is substituted with one to three substituents" means that the group referred to may or may not be substituted in order to fall within the scope of the invention. Thus the term "optionally substituted" is intended to mean that any one or more hydrogens on a designated atom can be replaced with a selection from the indicated group, provided that the designated atom's normal valence is not exceeded, and that the substitution results in a stable compound. When the substituent is keto (=O) then 2 hydrogens on the atom are replaced. There are one to three "optional substituents", unless otherwise indicated, and these substituents are independently selected from a group consisting of H; $N(R^{10})_2$; NO_2; halogen; aryl; O-C_{5-10} cyclo alkyl substituted with R^{10}; guanidino; urea; thio urea; amidino; para or meta phenoxy; piperidin-4-yloxy; 4-amino-cyclohexyloxy; 1-(1-Imino-ethyl)-piperidin-4-yloxy; 1-(1-Imino-ethyl)-pyrrolidin-3-yloxy; 2-Amino-3-methyl-butyryl; 4-Acetimidoylamino-cyclohexyloxy; 1-(1-Imino-ethyl)-pyrrolidin-2-ylmethoxy; 2-(2-Hydroxycarbonimidoyl-pyridin-3-yloxy)-ethoxy; 3,4-Dicyano-phenoxy; SC_{1-4} alkyl, S-aryl, O-C_{1-4} alkyl, $COOR^{10}$, OR^{10}, C(O)-pyrrolidine; $C(O)CH(NH_2)CH_2OH$; $C(O)CH(NH_2)CH_2Ph$; $C(O)CH(NH_2)CH_2COOH$; O-pyrrolidine; $C(O)-(CH_2)_{1-3}$-imidazole; SO_2-$N(alkyl)_2$; C(=N)-C_3; O-piperidine; 2-aminothiazol-5-ylmethoxy; O-CH_2-COOH; pyrrolidine-2-ylmethoxy; 2,4,6-triamino pyrimidin-5-ylmethoxy; NH-SO_2-alkyl; NHC_1-C_4 alkyl; $N(C_1-C_4)_2$ alkyl; CF_3; C_{2-10} alkenyl and C_{1-10} alkyl.

The term "alkyl", as used herein, is intended to include branched and straight chain saturated aliphatic hydrocarbon groups having from 1 to 14 or the specified number of carbon atoms, illustrative examples of which include, but are not limited to, methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, sec-butyl, t-butyl, n-pentyl, and

n-hexyl. "Alkenyl" is intended to include a branched or straight chain hydrocarbon group having one or more unsaturated carbon-carbon bonds which may occur in any stable point along the chain, such as ethenyl, propenyl, and the like. The term "alkelene" represents an alkyl group, as defined above, except that it has at least one center of unsaturation, i.e., a double bond. Illustrative examples are butene, propene, and pentene. The term "cycloalkyl", "cycloalkyl ring", "cycloalkyl radical" or "cyclic hydrocarbon" indicates a saturated or partially unsaturated three to fourteen carbon monocyclic or bicyclic hydrocarbon moiety which is optionally substituted with an alkyl group. Illustrative examples include cyclo propyl, cyclo hexyl, cyclo pentyl, and cyclo butyl. The term "alkoxy" as used herein represents $-OC_{1-6}$ alkyl.

The terms "Ar" and "aryl", as used herein, are intended to represent a stable substituted or unsubstituted (collectively also referred to as 'optionally substituted') six to fourteen membered mono-, bi- or tri-cyclic hydrocarbon radical comprising carbon and hydrogen atoms. Illustrative examples are phenyl (Ph), naphthyl, anthracyl groups, and piperanyl. It is also intended that the terms "carbocycle" and "carbocyclic" include "Ar", "aryl" as well as "cyclo alkyl" groups, which are defined above. "Halogen" or "halo", as used herein, represents Cl, Br, F or I.

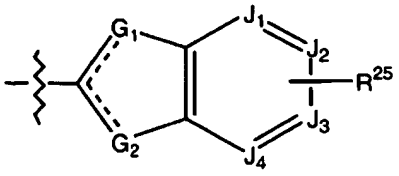
The term "heteroaryl" is intended to represent a stable 5 to 10 membered aryl group ("aryl" as defined above), wherein one or more of the carbon atoms is replaced by a hetero atom selected from N, O, and S. The hetero atoms can exist in their chemically allowed oxidation states. Thus a Sulfur (S) atom can exist as a sulfide, sulfoxide, or sulfone. Preferred heteroaryl groups are six membered ring systems comprising not more than 2 hetero atoms. Illustrative examples of preferred heteroaryl groups are thienyl, N-substituted succinimide, 3-(alkyl amino)-5,5-

dialkyl-2-cyclohexen-1-one, methyl pyridyl, alkyl theophylline, furyl, pyrrolyl, indolyl, pyrimidinyl, isoxazolyl, purinyl, imidazolyl, pyridyl, pyrazolyl, quinolyl, and pyrazinyl. The term "heterocycloalkyl" means a stable cyclo alkyl group containing from 5 to 14 carbon atoms wherein one or more of the carbon atoms is replaced by a hetero atom chosen from N, O and S. The hetero atoms can exist in their chemically allowed oxidation states. Thus Sulfur (S) can exist as a sulfide, sulfoxide, or sulfone. The heterocycloalkyl group can be completely saturated or partially unsaturated. Illustrative examples are piperidine, 1,4-dioxane, and morpholine.

As used herein the terms "heterocyclyl", "heterocyclic" and/or "het" are intended to represent a stable 5- to 7- membered monocyclic or 7- to 10- membered bicyclic heterocyclic ring which is saturated, partially unsaturated, or unsaturated (aromatic), which consists of carbon atoms and from one to 4 hetero atoms independently selected from a group consisting of N, O and S. The nitrogen and the sulfur hetero atoms can exist in their respective oxidized states. The heterocyclic ring may be attached to its pendent group at any hetero atom or carbon atom which results in a stable structure. The heterocyclic rings described herein may be substituted on a carbon or a nitrogen atom if the resulting compound is stable. The nitrogen in the heterocycle can exist in its quaternized form. It is preferred that when the total number of hetero atoms in the heterocycle exceeds 1, then the hetero atoms are not adjacent to one another. It is understood that the terms "heterocyclyl", "heterocyclic", and "het" include the terms "heteroaryl", "heterocycloalkyl" and "bicyclic heterocyclic ring structure" as described above.

Preferred "heterocyclyl", "heterocyclic" and/or "het" groups are selected from 1-(2-Hydroxymethyl-pyrrolidin-1-yl)-2,3-dimethyl-butan-1-one, 3-Pyridin-2-yl-propan-1-ol, N-(2,3-Dimethoxy-benzyl)-2-hydroxy-acetamide, 1-Methyl-2-m-tolyl-1H-benzoimidazole-5-carboxamidine, 2-Methyl-3,4,6,7-tetrahydro-imidazo[4,5-c]pyridine-5-carboxamidine, 2-Amino-3-hydroxy-1-(2-methyl-3,4,6,7-tetrahydro-imidazo[4,5-c]pyridin-5-yl)-propan-1-one, 2-Amino-1-(2-methyl-3,4,6,7-tetrahydro-imidazo[4,5-c]pyridin-5-yl)-ethane, 2-Methyl-4,5,6,7-tetrahydro-3H-imidazo[4,5-c]pyridine, N-o-Tolyl-methanesulfonamide, 2-Methyl-benzothiazole, 3-Amino-1-(2-hydroxymethyl-pyrrolidin-1-yl)-propan-1-one, 2-Hydroxy-1-(2-hydroxymethyl-pyrrolidin-1-yl)-ethanone, 2-(2-Hydroxy-ethyl)-indan-1,3-dione, 5-Fluoro-2-methyl-1H-benzoimidazole, 2-Methyl-1H-imidazo[4,5-c]pyridine, 2-Hydroxy-N-(2-morpholin-4-yl-ethyl)-acetamide, 2-Methyl-1H-imidazo[4,5-b]pyridine, 2-Amino-1-(3-methyl-piperidin-1-yl)-ethanone, 2-Methyl-1H-benzoimidazol-4-ol, 2-Pyridin-2-yl-ethanol, N-(3-Hydroxy-propyl)-2-phenyl-acetamide, N-(3-Hydroxy-propyl)-3-phenyl-propionamide, N-(3-Hydroxy-propyl)-benzamide, N-(2-Hydroxy-ethyl)-2-phenyl-acetamide, (4-Hydroxy-butyl)-carbamic acid tert-butyl ester, (2-Hydroxy-ethyl)-carbamic acid benzyl ester, (4-Hydroxy-piperidin-1-yl)-phenyl-methanone, 4-Bromo-2-methoxy-benzylamine, 3-Methoxy-5-trifluoromethyl-benzylamine, N-(3,5-Dimethoxy-benzyl)-acetamide, 2-Methyl-1H-benzoimidazole-5-carboxamidine, and 2-Hydroxy-N-naphthalen-1-yl-acetamide.

The following structural representations further illustrate the term "het":



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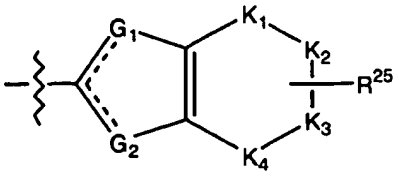

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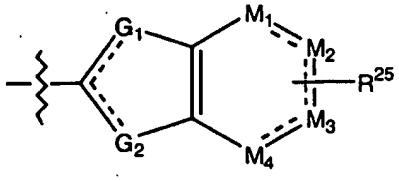

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wherein G_1 and G_2 independently at each occurrence represent $S(O)_{0-2}$, NH, N-R^{24}, O, CR^{10}, or CHR^{10}; J_1, J_2, J_3, and J_4 independently represent CR^{10} or N, wherein at least two of J_1, J_2, J_3, and J_4 represent CH ; K_1, K_2, K_3 and K_4 independently represent -NHR^{10}, -NHR^{24}, -CHR^{10}, -CH-C(=NH)-NH_2, or N-C(=NH)-NH_2 wherein at least two of K_1, K_2, K_3 and K_4 represent CH_2 ; M_1, M_2, M_3 and M_4 independently represent -NHR^{10}, -NHR^{24}, -CHR^{10}, -CH-C(=NH)-NH_2, or N-C(=NH)-NH_2, wherein at least two of M_1, M_2, M_3 and M_4 represent CH or CH_2; and R^{25} represents H, halogen, -C_{1-6} alkyl, -NO_2, NHR^{10}, NH-SO_2-R^{10}, -OH, C_{1-6} alkoxy, amidino, guanidino, -$COOR^{10}$, or -$CONHR^{10}$. The variables R^{10} and R^{24} are as defined earlier. The dashed lines indicate optional unsaturation without violating the valency rules.

The term "basic group" as used under R^7 and R^8, defined earlier, is intended to represent amidino, guanidino, -C(=NH)$N(R^{10})_2$, 2-imidazoline, -N-amidinomorpholine, N-amidino piperidine, 4-hydroxy-N-amidino piperidine, N-amidino pyrrolidine, tetrahydro pyrimidine, and thiazolidin-3-yl-methylideneamine. The compounds of the present invention were named using the "Autonom", a Beilstein Commander 2.1 Application, distributed by Beilstein.

The term "acylatable group" as used herein represents a group which is capable of reacting with an acylating group to form an amido group. Illustrative examples of acylatable groups are primary or secondary amino, guanidino and amidino.

The term "acylating agent" as used herein represents a chemical agent which is capable of reacting with an acylatable group to form an amido group. Illustrative examples of an acylating agent are acid chloride and *N*-methylpyrrolidone.

The term "acetamide" as used herein represents a reagent that comprises an acetamide group. Illustrative examples of an acetamide are alkyl acetamide, dialkyl acetamide, dimethyl acetamide, dialkyl propionamide, and diethyl acetamide. The acetamide functions as a solvent and a base in the process of the present invention.

The term "natural amino acid", as used herein is intended to represent the twenty naturally occurring amino acids in their 'L' form, which are some times also referred as 'common amino acids', a list of which can be found in *Biochemistry*, Harper & Row Publishers, Inc. (1983). The term "unnatural amino acid", as used herein, is intended to represent the 'D' form of the twenty naturally occurring amino acids described above. It is further understood that the term unnatural amino acid includes homologues of the natural amino acids, and synthetically modified form of the natural amino acids. The synthetically modified forms include amino acids having alkylene chains shortened or lengthened by up to two carbon atoms, amino acids comprising optionally substituted aryl groups, and amino acids comprised halogenated groups, preferably halogenated alkyl and aryl groups.

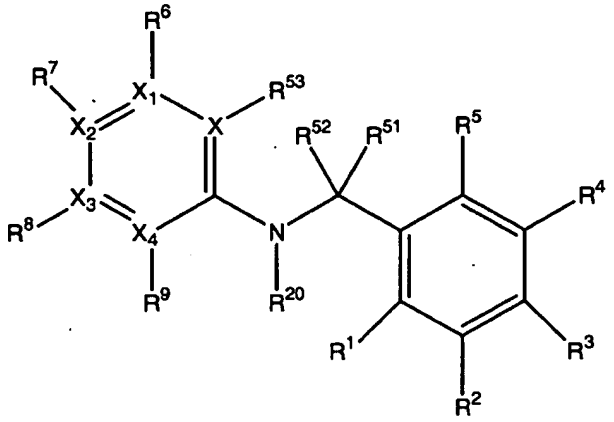
The term "natural amino acid side chain" is intended to represent a natural amino acid ("natural amino acid" as defined above) wherein a keto (C=O) group

replaces the carboxylic acid group in the amino acid. Thus, for example, an alanine side chain is C(=O)-CH(NH_2)-CH_3; a valine side chain is C(=O)-CH(NH_2)-$CH(CH_3)_2$; and a cysteine side chain is C(=O)-CH(NH_2)-CH_2-SH. The term "unnatural amino acid side chain" is intended to represent an unnatural amino acid ("unnatural amino acid" as defined above) wherein a keto (C=O) group replaces the carboxylic acid group forming unnatural amino acid side chains similar to ones illustrated under the definition of "natural amino acid side chain" above.

It thus follows that a "N-natural amino acid side chain" substituent and "N-unnatural amino acid side chain" substituent, which can represent Q, Q^1, Q^2, Q^3, L^1, L^2, L^3 and L^4, is a group wherein the nitrogen atom (N) is the annular ring atom substituted with a natural or unnatural amino acid side chain (natural or unnatural amino acid side chain is a defined above). The point of attachment between the nitrogen atom and the natural or unnatural amino acid side chain is at the keto (C=O) group of the respective amino acids. Thus a N-natural amino acid, i.e., N-cysteine, is N-C(=O)-CH(NH_2)-CH_2-SH.

CLAIMS:

1. A compound of Formula I:



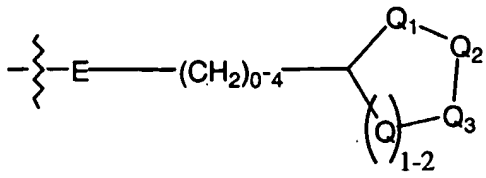
Formula I

its prodrug form or pharmaceutically acceptable salts thereof, wherein:

R^1 represents OH, COOH, COO-C_{1-4} alkyl, CH_2OR^{10}, SO_2-OH, O-SO_2-OH, O-SO_2-OC_{1-4} alkyl, $OP(O)(OH)_2$, or OPO_3C_{1-4} alkyl;

R^2, R^3, R^4, and R^5 independently at each occurrence represent H, SH, OR^{10}, halogen, $COOR^{10}$, $CONR^{11}R^{12}$, optionally substituted aryl, optionally substituted heterocyclyl, C_{4-14} cycloalkyl-C_{1-4} alkyl, C_{1-4} alkyl aryl, optionally substituted C_{1-14} straight chain, branched or cyclo alkyl, $NR^{10}R^{24}$, $(CH_2)_{1-4}$-$NR^{33}R^{34}$, $(CH_2)_{1-4}$-$COOR^{33}$, O-$(CH_2)_{1-3}$-CO-het, O-$(CH_2)_{1-2}$-NH-CO-aryl, O-$(CH_2)_{0-2}$-NR^{10}-CO-$NR^{10}R^{33}$, O-$(CH_2)_{0-2}$-C(O)-$NR^{33}R^{34}$, O-$(CH_2)_{1-4}$-$COOR^{10}$, O-$(CH_2)_{1-3}$-het-R^{32}, O-optionally substituted cycloalkyl, O-$(CH_2)_{1-4}$-NR^{10}-COO-*t*-butyl, O-$(CH_2)_{1-4}$-$NR^{10}R^{33}$, O-$(CH_2)_{1-4}$-NR^{10}-C(O)-C_{0-3}-alkyl-optionally substituted aryl, O-$(CH_2)_{0-6}$-

optionally substituted aryl, $(CH_2)_{1-4}$-NH-C(O)O-$(CH_2)_{1-4}$-Ph$R^{13}R^{14}$, NO_2, O-$(CH_2)_{0-4}$-C(O)-NH-tetrahydro carboline, SO_3H, $CH(OH)COOR^{10}$, $NR^{10}R^{28}$, O-$(CH_2)_{1-3}$-optionally substituted het, CH_2COOCH_3, CH=CH-$COOCH_3$,



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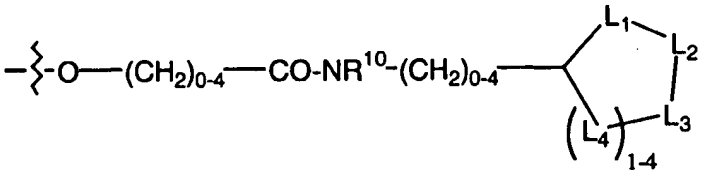
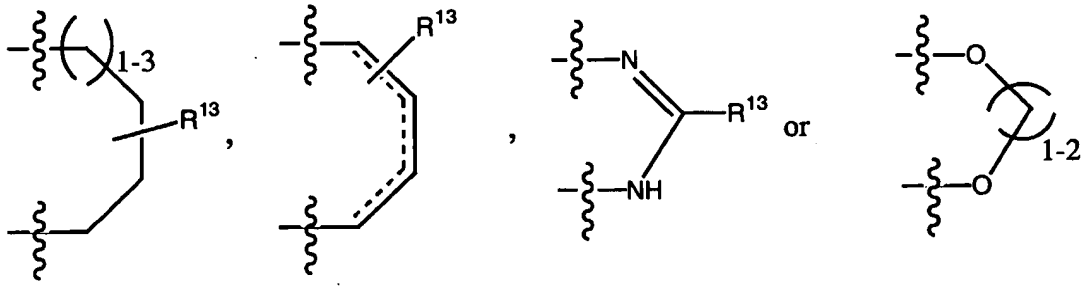


;

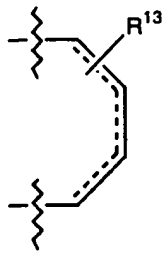
alternatively R^2 and R^3, R^3 and R^4, or R^4 and R^5 taken together form



;

R^6, R^9 and R^{53} independently at each occurrence represents H, halogen, cyano, C_{1-4} alkyl, C_{1-4} halogenated alkyl, NO_2, O-aryl or OR^{11};

alternatively R^6 and R^{53} taken together form



;

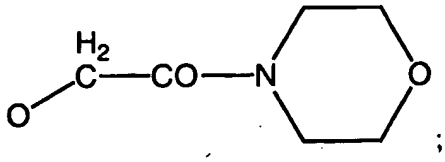
R^7 and R^8 independently at each occurrence represent OH, CF_3, H, COOH, NO_2, C_{1-4} alkyl, OC_{1-4} alkyl, or O-aryl, halogen, cyano, or a basic group selected from guanidino, $NH(CH=NH)NH_2$, $C(=NH)N(R^{10})_2$, $C(=NH)$-NH-NH_2, $C(=O)N(R^{10})_2$, 2-imidazoline, N-amidinomorpholine, N-amidino piperidine, 4-hydroxy-N-amidino piperidine, N-amidino pyrrolidine, tetrahydro pyrimidine, $C(O)CH_2NH_2$, $C(O)NHCH_2CN$, $NHCH_2CN$, and thiazolidin-3-yl-methylideneamine; with the proviso that only one of R^7 and R^8 represent a basic group;

R^{10} independently at each occurrence represents H, $(CH_2)_{0-2}$-aryl, C_{1-4} halo alkyl, or C_{1-14} straight chain, branched or cyclo alkyl, and alternatively, when one atom is substituted with two R^{10} groups, the atom along with the R^{10} groups can form a five to 10 membered ring structure;

X_1, X_2, X_3 and X_4 independently at each occurrence represent a carbon or a nitrogen atom;

R^{11} and R^{12} independently at each occurrence represent H or C_{1-4} alkyl;

R^{13} represents H, OH, OC_{1-4} alkyl, OAr, OC_{5-10} cycloalkyl, OCH_2CN, $O(CH_2)_{1-2}NH_2$, OCH_2COOH, OCH_2COO-C_{1-4} alkyl or



;

R^{20} represents H or OH;

R^{24} represents R^{10}, $(CH_2)_{1-4}$-optionally substituted aryl, $(CH_2)_{0-4}OR^{10}$, $CO-(CH_2)_{1-2}-N(R^{10})_2$, $CO(CH_2)_{1-4}-OR^{10}$, $(CH_2)_{1-4}-COOR^{10}$, $(CH_2)_{0-4}-N(R^{10})_2$, SO_2R^{10}, COR^{10}, $CON(R^{10})_2$, $(CH_2)_{0-4}$-aryl-$COOR^{10}$, $(CH_2)_{0-4}$-aryl-$N(R^{10})_2$, or $(CH_2)_{1-4}$-het-aryl;

R^{28} represents $(CH_2)_{1-2}$-Ph-O-$(CH_2)_{0-2}$-het-R^{30}, C(O)-het, CH_2-Ph-CH_2-het-$(R^{30})_{1-3}$; $(CH_2)_{1-4}$-cyclohexyl-R^{31}, CH_2-Ph-O-Ph-$(R^{30})_{1-2}$, CH_2-(CH_2OH)-het-R^{30}, CH_2-Ph-O-cycloalkyl-R^{31}, CH_2-het-C(O)-CH_2-het-R^{30}, or CH_2-Ph-O-(CH_2)-O-het-R^{30};

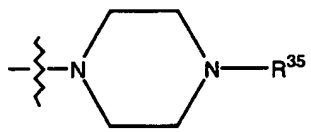
R^{30} represents $SO_2N(R^{10})_2$, H, NHOH, amidino, or $C(=NH)CH_3$;

R^{31} represents R^{30}, amino-amidino, NH-$C(=NH)CH_3$ or R^{10};

R^{32} represents H, C(O)-CH_2-NH_2, or C(O)-$CH(CH(CH_3)_2)$-NH_2;

R^{33} and R^{34} independently at each occurrence represent R^{10}, $(CH_2)_{0-4}$-Ar, optionally substituted aryl, $(CH_2)_{0-4}$ optionally substituted heteroaryl, $(CH_2)_{1-4}$-CN, $(CH_2)_{1-4}-N(R^{10})_2$, $(CH_2)_{1-4}$-OH, $(CH_2)_{1-4}-SO_2-N(R^{10})_2$;

alternatively, R^{33} and R^{34} along with the nitrogen atom that they are attached to forms a 4 to 14 atom ring structure selected from tetrahydro-1H-carboline; 6,7-Dialkoxyoxy-2-substituted 1,2,3,4-tetrahydro-isoquinoline,



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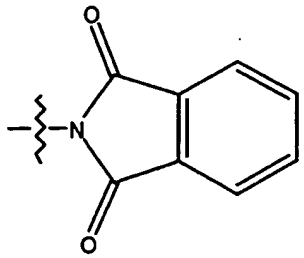


;

R^{35} represents R^{10}, SO_2-R^{10}, COR^{10}, or $CONHR^{10}$;

E represents a bond, $S(O)_{0-2}$, O or NR^{10};

Q, Q^1, Q^2, Q^3, L^1, L^2, L^3 and L^4 independently at each occurrence represent N-natural or unnatural amino acid side chain, CHR^{10}, O, NH, $S(O)_{0-2}$, N-C(O)-NHR^{10}, SO_2-$N(R^{10})_2$, N-C(O)-NH-$(CH_2)_{1-4}$-R^{26}, NR^{10}, N-heteroaryl, N-C(=NH)-NHR^{10}, or N-C(=NH)C_{1-4} alkyl;

R^{26} represents OH, NH_2, or SH;

R^{51} and R^{52} independently represent COOH, CH_2OH, CH_2COOH, COOR, CH_2COOR, alkyl or CO-NH_2; alternatively

R^{51} and R^{52} taken together represent =O, =S, $=CH_2$ or $=NR^{10}$;

R^{53} represents H, halogen, cyano, C_{1-4} alkyl, C_{1-4} halogenated alkyl, NO_2, O-aryl or OR^{11};

with the proviso that at least two of X_1, X_2, X_3 and X_4 represent a carbon atom, and when any of X_1, X_2, X_3 and X_4 represent a nitrogen atom the corresponding substituent does not exist.

2. A compound of Claim 1 wherein

R^1 represents OH or COOH;

R^{20} represents H;

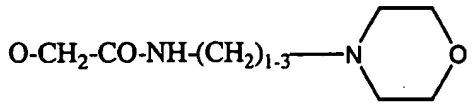
R^{51} and R^{52} taken together form =O; and

X_1, X_2, X_3, and X_4 represent C.

3. A compound of Claim 2 wherein:

R^2 represents halo, H, NH-CO-Ph, *i*-propyl, OH, OCH_3, OC_2H_5, CH(OH)COOH, O-*I*-propyl, SO_3H, NH_2, CH(OH)$COOC_{1-2}$ alkyl, CH_3, NO_2 or Ph;

R^3 represents H, OH, NH_2 OC_{1-4} alkyl, C_{1-4} alkyl, $NHCH_3$, O-$(CH_2)_{1-3}$-OCO-C_{1-2} alkyl, NH-C(O)C_{1-2} alkyl, O-$(CH_2)_{1-2}$-CO-NH_2, Ph, $NHCOCF_3$, N=CH-$N(CH_3)_2$, O-CH_2-CO-NH-$(CH_2)_{1-3}$-Ph,



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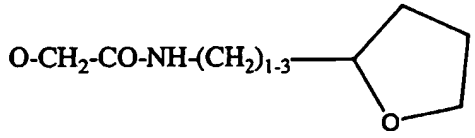
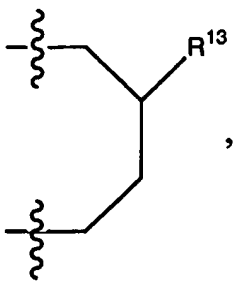


;

R^4 represents H, C_{1-4} alkyl, halogen, *i*-propyl, OH, NH_2 3-nitro-phen-1-yl, NH-CO-CH_3, CH_2-NH-$(CH_2)_3$-Ph, 2,4-difluoro-phen-1-yl, $NHCOCF_3$, benzo[1,3]dioxol-5-yl, 4-Carbamimidoyl-phenylazo, 3-Hydroxy-4-carboxyl-phenylsulfanyl; 1,3-Dioxo-indan-2-yl, or toluene-4-sulfonylamino;

R^5 represents H or OH;

alternatively, R^2 and R^3, R^3 and R^4, or R^4 and R^5 can be taken together to form



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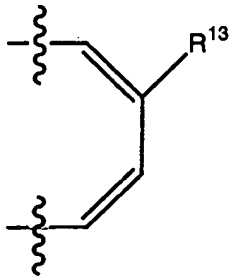

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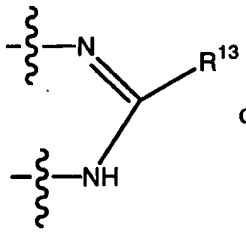

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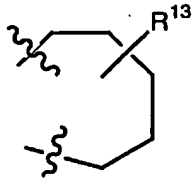


;

R^6 represents H;

R^7 represents C(=NH)-NH_2 or NH-C(=NH)-NH_2;

R^8 represents H or halogen; and

R^9 represents H.

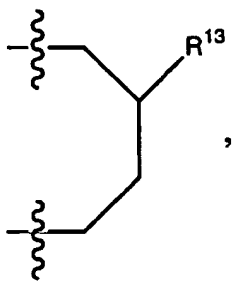
4. A compound of claim 3 wherein

R^2 represents halo, H, NH-CO-Ph, *i*-propyl, OH, CH_3, or NO_2;

R^3 represents H, OH, NH_2 OC_{1-2} alkyl, C_{1-4} alkyl, O-$(CH_2)_{1-3}$-OCO-C_{1-2} alkyl, NH-C(O)CH_3, O-CH_2-CO-NH_2, Ph, $NHCOCF_3$, N=CH-$N(CH_3)_2$, O-CH_2-CO-NH-$(CH_2)_2$-Ph;

R^4 represents H, CH_3, methoxy, halogen, *i*-propyl, 3-nitro-phen-1-yl, $NHCOCF_3$, benzo[1,3]dioxol-5-yl, $NHCOCH_3$, 4-Carbamimidoyl-phenylazo, 3-Hydroxy-4-carboxyl-phenylsulfanyl or 1,3-Dioxo-indan-2-yl;

alternatively, R^2 and R^3, R^3 and R^4, or R^4 and R^5 can be taken together to form



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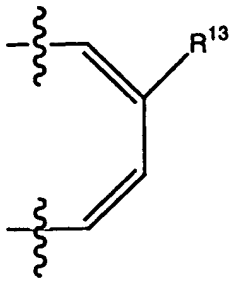

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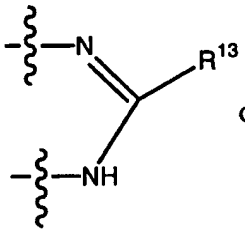

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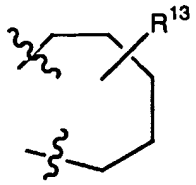
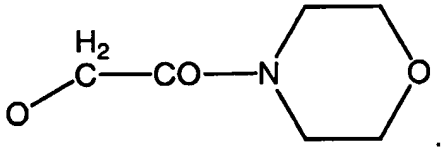


; and

R^{13} represents C_{1-2} alkyl, OH, O$(CH_2)_{1-2}$-NH_2, H, or

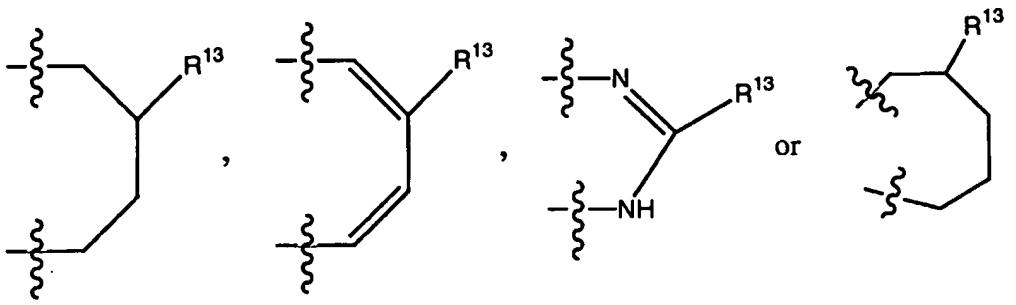


5. A compound of Claim 4 wherein

R^3 represents H, OH, NH_2 OC_{1-2} alkyl, C_{1-4} alkyl, O-CH_2-OCO-CH_3, NH-C(O)CH_3, O-CH_2-CO-NH_2;

R^4 represents H, CH_3, halogen, *i*-propyl, benzo[1,3]dioxol-5-yl, or 1,3-Dioxo-indan-2-yl;

alternatively, R^2 and R^3, R^3 and R^4, or R^4 and R^5 can be taken together to form



6. A compound of Claim 5 wherein

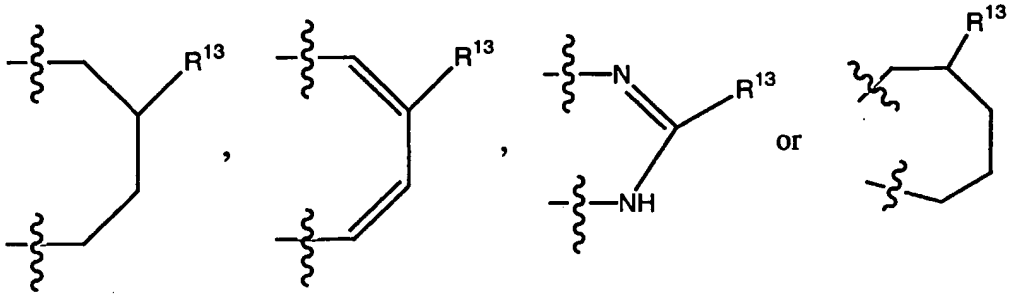
R^2 represents H or halogen;

R^3 represents H, OH or NH_2;

R^4 represents H, CH_3, halogen or benzo[1,3]dioxol-5-yl;

R^5 represents H; or

R^3 and R^4 or taken together to form



7. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of (i) a compound; or (ii) a pharmaceutically acceptable salt of a compound of Claim 1.

8. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a compound or a pharmaceutically acceptable salt of a compound of Claim 4.

9. A method for treating or preventing a thromboembolic disorder, comprising administering to a patient in need thereof a therapeutically effective amount of a compound according to Claim 4 or a pharmaceutically acceptable salt thereof.

10. A compound of Claim 6, wherein the compound is selected from:

N-(4-Carbamimidoyl-phenyl)-2-hydroxy-3-iodo-5-methyl-benzamide;

3,5-Dibromo-N-(4-carbamimidoyl-phenyl)-2,4-dihydroxy-benzamide;

5-Bromo-N-(4-carbamimidoyl-phenyl)-2,4-dihydroxy-3-iodo-benzamide;

3-Hydroxy-naphthalene-2-carboxylic acid (6-guanidino-pyridin-3-yl)-amide; and

3-Hydroxy-7-methoxy-naphthalene-2-carboxylic acid (4-guanidino-phenyl)-amide.

11. A compound of Claim 1 wherein

R^1 represents OH or COOH;

R^{20} represents H;

R^{51} and R^{52} taken together form =O;

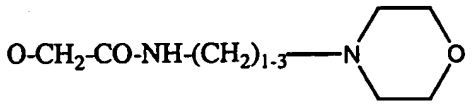
X_1 represents N; and

X_2, X_3, and X_4 represent C.

12. A compound of Claim 1 wherein

R^2 represents halo, H, NH-CO-Ph, *i*-propyl, OH, CH_3, NO_2 or Ph;

R^3 represents H, OH, NH_2 OC_{1-4} alkyl, C_{1-4} alkyl, $O-(CH_2)_{1-3}-OCO-C_{1-2}$ alkyl, $NH-C(O)C_{1-2}$ alkyl, $O-(CH_2)_{1-2}-CO-NH_2$, Ph, $NHCOCF_3$, $N=CH-N(CH_3)_2$, $O-CH_2-CO-NH-(CH_2)_{1-3}-Ph$,



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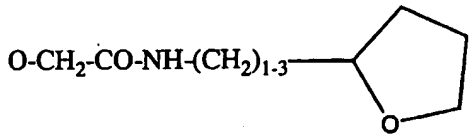
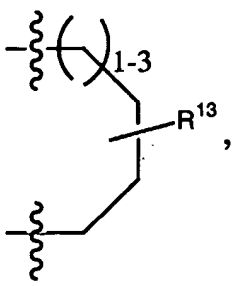


;

R^4 represents H, C_{1-4} alkyl, halogen, *i*-propyl, OH, NH_2 3-nitro-phen-1-yl, NH-CO-CH_3, CH_2-NH-$(CH_2)_3$-Ph, 2,4-difluoro-phen-1-yl, $NHCOCF_3$, benzo[1,3]dioxol-5-yl, 4-Carbamimidoyl-phenylazo, 3-Hydroxy-4-carboxyl-phenylsulfanyl; 1,3-Dioxo-indan-2-yl, or toluene-4-sulfonylamino;

R^5 represents H or OH;

alternatively, R^2 and R^3, R^3 and R^4, or R^4 and R^5 can be taken together to form



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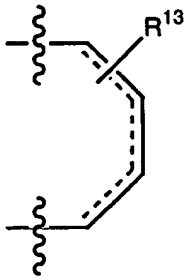

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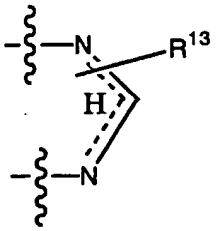

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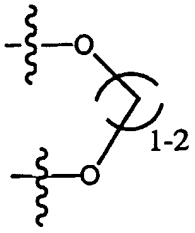


;

R^6 represents H;

R^7 represents C(=NH)-NH_2 or NH-C(=NH)-NH_2;

R^8 represents H or halogen; and

R^9 represents H.

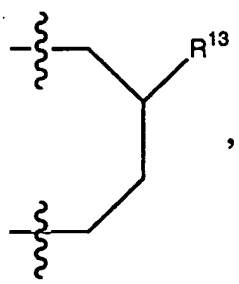
13. A compound of claim 12 wherein

R^2 represents halo, H, NH-CO-Ph, *i*-propyl, OH, CH_3, or NO_2;

R^3 represents H, OH, NH_2 OC_{1-2} alkyl, C_{1-4} alkyl, O-$(CH_2)_{1-3}$-OCO-C_{1-2} alkyl, NH-C(O)CH_3, O-CH_2-CO-NH_2, Ph, $NHCOCF_3$, N=CH-$N(CH_3)_2$, O-CH_2-CO-NH-$(CH_2)_2$-Ph;

R^4 represents H, CH_3, methoxy, halogen, *i*-propyl, 3-nitro-phen-1-yl, $NHCOCF_3$, benzo[1,3]dioxol-5-yl, $NHCOCH_3$, 4-Carbamimidoyl-phenylazo, 3-Hydroxy-4-carboxyl-phenylsulfanyl or 1,3-Dioxo-indan-2-yl;

alternatively, R^2 and R^3, R^3 and R^4, or R^4 and R^5 can be taken together to form



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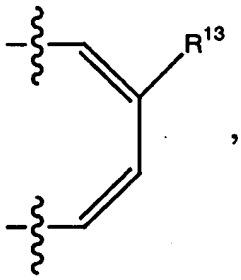

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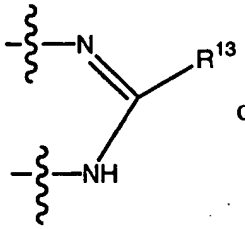

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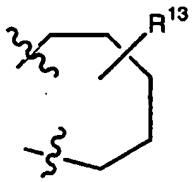
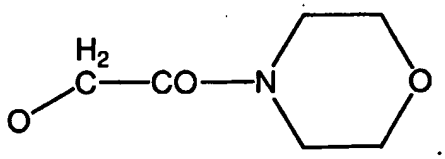


; and

R^{13} represents C_{1-2} alkyl, OH, O$(CH_2)_{1-2}$-NH_2, H, or

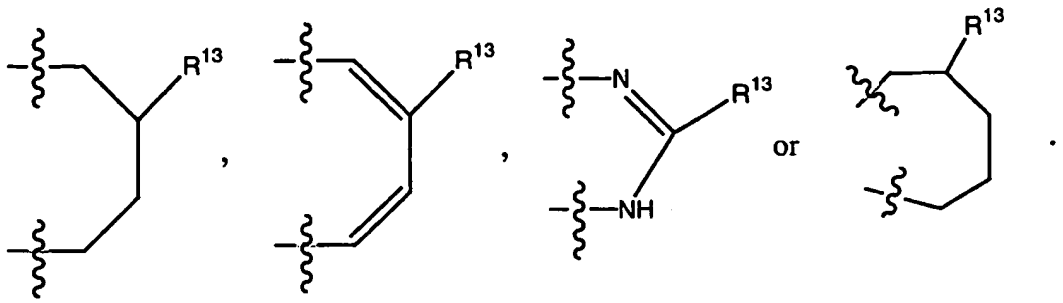


14. A compound of Claim 13 wherein

R^3 represents H, OH, NH_2 OC_{1-2} alkyl, C_{1-4} alkyl, O-CH_2-OCO-CH_3, NH-C(O)CH_3, O-CH_2-CO-NH_2;

R^4 represents H, CH_3, halogen, *i*-propyl, benzo[1,3]dioxol-5-yl, or 1,3-Dioxo-indan-2-yl;

alternatively, R^2 and R^3, R^3 and R^4, or R^4 and R^5 can be taken together to form



15. A compound of Claim 14 wherein

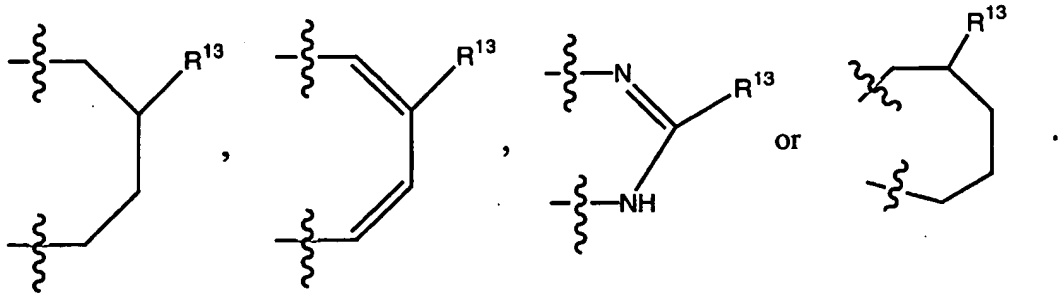
R^2 represents H or halogen;

R^3 represents H, OH or NH_2;

R^4 represents H, CH_3, halogen or benzo[1,3]dioxol-5-yl;

R^5 represents H; and

R^3 and R^4 or taken together to form



16. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a compound or a pharmaceutically acceptable salt of a compound of Claim 10.

17. A pharmaceutical composition comprising a pharmaceutically acceptable carrier and a therapeutically effective amount of a compound according to Claim 13 or a pharmaceutically acceptable salt thereof.

18. A method for treating or preventing a thromboembolic disorder, comprising administering to a patient in need thereof a therapeutically effective amount of a compound according to Claim 13 or a pharmaceutically acceptable salt thereof.

19. A method for treating cancer in mammals comprising administering a therapeutically effective amount of a compound according to Claim 13.

20. A process for selectively acylating an amino group, said process comprising treating a molecule comprising an amino group with an acylating agent in the presence of an acetamide to yield a compound with an acylated amino group.

21. A process of Claim 20 wherein the amino group is selectively acylated in the presence of another acylatable group.

22. A process of Claim 21 wherein the acylatable group is selected from an optionally substituted amino ketone, alkyl amidino, alkyl guanidino, C(=NH)NH-NH_2, aryl-$(CH_2)_{0-4}$-NHR^{10}, amidino and guanidino.

23. A process of Claim 22 wherein the acylating agent comprises an acid halide group.

24. A process of Claim 23 wherein the acetamide is an alkyl or dialkyl acetamide.

25. A process of Claim 24 wherein the acetamide is selected from a group consisting of DMA, diethyl acetamide, dimethyl propionamide, diethyl propionamide and N-methylpyrrolidinone.

26. A process of Claim 25 wherein the process is carried out at a temperature ranging from about 25°C to about 50°C.

27. A process of Claim 26 wherein the acylating agent is a protected salicylic acid chloride selected from acetic acid 2-chlorocarbonyl-phenyl ester and 2-benzyloxy-benzoyl chloride.

28. A method for treating or preventing a cancer related disorder, comprising administering to a patient/ mammal in need thereof a therapeutically effective amount of a compound of Claim 1 or a pharmaceutically acceptable salt thereof.

29. A method for treating or preventing a cancer related disorder, comprising administering to a patient/ mammal in need thereof a therapeutically effective amount of a compound of Claim 3 or a pharmaceutically acceptable salt thereof.

30. A method for treating or preventing a cancer related disorder, comprising administering to a patient/ mammal in need thereof a therapeutically effective amount of a compound of Claim 12 or a pharmaceutically acceptable salt thereof.

31. A method for treating or preventing a cancer related disorder, comprising administering to a patient/ mammal in need thereof a therapeutically effective amount of a compound of Claim 15 or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/ 00/34211

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C257/18 C07C279/18 A61K31/155 A61P7/02 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, PAJ, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Derwent Publications Ltd., London, GB; AN 1999-508614 XP002161780 "New amidino compound useful for treating..." & WO 99 41231 A (ONO PARMACEUTICALS CO LTD.), 19 August 1999 (1999-08-19) abstract & DATABASE REGISTRY [Online] CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; RN 239457-45-5 and 239457-46-6, ---	1,2,7,9, 11,18
A	WO 99 05096 A (ABBOTT LAB) 4 February 1999 (1999-02-04) the whole document ---	1-19, 28-31
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 March 2001	28-05-01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Janus, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 576 343 A (NAGAHARA TAKAYASU ET AL) 19 November 1996 (1996-11-19) cited in the application the whole document	1-19, 28-31
A	EP 0 635 492 A (LILLY CO ELI) 25 January 1995 (1995-01-25) examples 39,40	1-19, 28-31
A	EP 0 703 216 A (ONO PHARMACEUTICAL CO) 27 March 1996 (1996-03-27) tables 1,3,8,10	1-19, 28-31

INTERNATIONAL SEARCH REPORT

International application No.
CT/US 00/34211

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 9, 18, 19 and 28-31 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound.

2. [X] Claims Nos.: 1-19 and 28-31 (all in part)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1 - 19, 28 - 31

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-19 and 28-31 (all in part)

Present claims 1-6 and 10-15 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT is to be found, however, for only a very small proportion of the compounds claimed. The same applies to the compositions claimed in claims 7, 8, 16 and 17 and to the methods of claims 9, 18, 19 and 28-31. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds or formula I wherein R1 is OH, R51 and R52 together are =O, R20 is H, X, X2, X3 and X4 are C, R9 is H, and R7 is amidinyl or guanidinyl.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-19, 28-31

Compounds of formula (I), their use in pharmaceutical compositions, and in the treatment of thromboembolic disorders and of cancer.

2. Claims: 20-27

Process for the acylation of an amino group.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/ 00/34211

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		EP 1078917 A	28-02-2001
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/ .00/34211

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(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(19) World Intellectual Property Organization
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(43) International Publication Date
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PCT

(10) International Publication Number
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(51) International Patent Classification[7]: C07C 257/18, 279/18, A61K 31/155, A61P 7/02, 35/00

(21) International Application Number: PCT/US00/34211

(22) International Filing Date:
14 December 2000 (14.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/170,916 15 December 1999 (15.12.1999) US

(71) Applicant *(for all designated States except US)*: AXYS PHARMACEUTICALS, INC. [US/US]; 180 Kimball Way, South San Francisco, CA 94080 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: ALLEN, Darin, Arthur [US/US]; 855 Mountain View Drive, #8, Daly City, CA 94014 (US). MCGEE, Danny, Peter, Claude [CA/US]; 20 S. Grant Street, San Mateo, CA 94401 (US). SPENCER, Jeffrey, R. [US/US]; 8 Baycrest Way, South San Francisco, CA 94080 (US).

(74) Agents: KATHARDEKAR, Vinit, G. et al.; Axys Pharmaceuticals, Inc., 180 Kimball Way, South San Francisco, CA 94080 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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(48) Date of publication of this corrected version:
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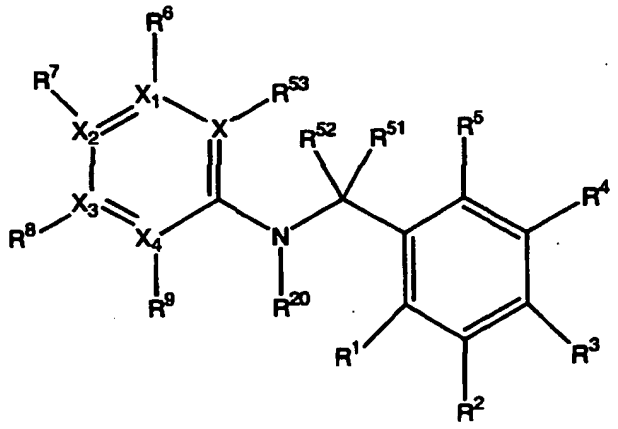
[Continued on next page]

(54) Title: SALICYLAMIDES AS SERINE PROTEASE AND FACTOR XA INHIBITORS



(I)

(57) Abstract: The present invention provides novel compounds of Formula (I), its prodrug forms, or pharmaceutically acceptable salts thereof. The compounds of this invention are inhibitors of serine proteases, Urokinase (uPA), Factor Xa (FXa), and/or Factor VIIa (FVIIa), and have utility as anti cancer agents and/or as anticoagulants for the treatment or prevention of thromboembolic disorders in mammals. The present invention also provides a process for the selective acylation of an amino group.

WO 01/44172 A1

(15) **Information about Correction:**
see PCT Gazette No. 29/2001 of 19 July 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(51) International Patent Classification[7]: C07C 257/18, 279/18, A61K 31/155, A61P 7/02, 35/00

(21) International Application Number: PCT/US00/34211

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14 December 2000 (14.12.2000)

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(30) Priority Data:
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(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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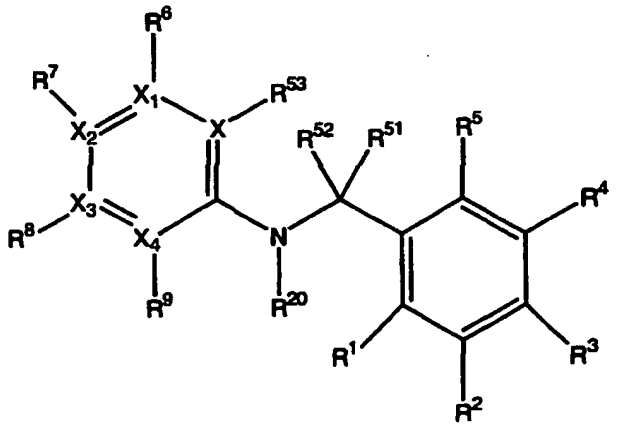
(54) Title: SALICYLAMIDES AS SERINE PROTEASE AND FACTOR XA INHIBITORS



(I)

(57) Abstract: The present invention provides novel compounds of Formula (I), its prodrug forms, or pharmaceutically acceptable salts thereof. The compounds of this invention are inhibitors of serine proteases, Urokinase (uPA), Factor Xa (FXa), and/or Factor VIIa (FVIIa), and have utility as anti cancer agents and/or as anticoagulants for the treatment or prevention of thromboembolic disorders in mammals. The present invention also provides a process for the selective acylation of an amino group.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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(51) International Patent Classification[7]: C07D

(21) International Application Number: PCT/US00/28458

(22) International Filing Date: 13 October 2000 (13.10.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/418,546 14 October 1999 (14.10.1999) US

(71) Applicant: ENTREMED, INC. [US/US]; 9640 Medical Center Drive, Rockville, MD 20850 (US).

(72) Inventors: GREEN, Shawn, J.; 9416 Gamba Court, Vienna, VA 22182 (US). PAPATHANASSIU, Adonia, E.; 1220 East-West Highway, Apartment 811, Silver Spring, MD 20910 (US).

(74) Agents: GREENE, Jamie, L. et al.; Kilpatrick Stockton, LLP, 2400 Monarch Tower, 3424 Peachtree Road, N.E., Atlanta, GA 30326 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/27079 A2

(54) Title: COMPOSITIONS AND METHODS OF USE OF LIGANDS THAT BIND COMPONENTS OF THE BLOOD COAGULATION/CLOTTING PATHWAY FOR THE TREATMENT OF CANCER AND ANGIOGENIC-BASED DISEASE

(57) Abstract: Compositions and methods effective in inhibiting abnormal or undesirable cell proliferation, particularly endothelial cell proliferation and angiogenesis related to neovascularization and tumor growth are provided. The compositions comprise naturally occurring, or synthetic proteins, peptides, or protein fragments capable of binding to components of the blood coagulation pathway. The compositions may be administered using a pharmaceutically acceptable carrier. The methods involve administering to a human or animal the composition described herein in a dosage sufficient to inhibit cell proliferation, particularly endothelial cell proliferation. The methods are useful for treating diseases and processes mediated by undesired and uncontrolled cell proliferation, such as cancer, particularly by inhibiting angiogenesis. Administration of the composition to a human or animal having prevascularized metastasized tumors is useful for preventing the growth or expansion of such tumors.

COMPOSITIONS AND METHODS OF USE OF LIGANDS THAT BIND COMPONENTS OF THE BLOOD COAGULATION/CLOTTING PATHWAY FOR THE TREATMENT OF CANCER AND ANGIOGENIC-BASED DISEASE

FIELD OF THE INVENTION

The present invention relates to compositions and methods for the inhibition of cellular proliferation. More particularly, the present invention relates to the use of ligands that bind to components of the blood coagulation/clotting pathway, and inhibit angiogenesis and angiogenesis-related diseases.

BACKGROUND OF THE INVENTION

Cellular proliferation is a normal ongoing process in all living organisms and is one that involves numerous factors and signals that are delicately balanced to maintain regular cellular cycles. The general process of cell division is one that consists of two sequential processes: nuclear division (mitosis), and cytoplasmic division (cytokinesis). Because organisms are continually growing and replacing cells, cellular proliferation is a central process that is vital to the normal functioning of almost all biological processes. Whether or not mammalian cells will grow and divide is determined by a variety of feedback control mechanisms, which include the availability of space in which a cell can grow, and the secretion

of specific stimulatory and inhibitory factors in the immediate environment.

When normal cellular proliferation is disturbed or somehow disrupted, the results can affect an array of biological functions. Disruption of proliferation could be due to a myriad of factors such as the absence or overabundance of various signaling chemicals or presence of altered environments. Some disorders characterized by abnormal cellular proliferation include cancer, abnormal development of embryos, improper formation of the corpus luteum, difficulty in wound healing as well as malfunctioning of inflammatory and immune responses.

Cancer is characterized by abnormal cellular proliferation. Cancer cells exhibit a number of properties that make them dangerous to the host, often including an ability to invade other tissues and to induce capillary ingrowth, which assures that the proliferating cancer cells have an adequate supply of blood. One of the defining features of cancer cells is that they respond abnormally to control mechanisms that regulate the division of normal cells and continue to divide in a relatively uncontrolled fashion until they kill the host.

Angiogenesis and angiogenesis-related diseases are closely affected by cellular proliferation. As used herein, the term "angiogenesis" means the generation of new blood vessels into a tissue or organ. Under normal physiological conditions, humans or animals undergo angiogenesis only in very specific restricted situations. For example, angiogenesis is normally observed in wound healing, fetal and embryonal development and formation of the corpus luteum, endometrium and placenta. The term "endothelium" is defined herein as a thin layer of flat cells that lines serous cavities, lymph vessels, and blood vessels. These cells are defined herein as "endothelial cells". The term "endothelial inhibiting activity" means the capability of a molecule to inhibit angiogenesis in general.

The inhibition of endothelial cell proliferation also results in an inhibition of angiogenesis.

Both controlled and uncontrolled angiogenesis are thought to proceed in a similar manner. Endothelial cells and pericytes, surrounded by a basement membrane, form capillary blood vessels. Angiogenesis begins with the erosion of the basement membrane by enzymes released by endothelial cells and leukocytes. The endothelial cells, which line the lumen of blood vessels, then protrude through the basement membrane. Angiogenic stimulants induce the endothelial cells to migrate through the eroded basement membrane. The migrating cells form a "sprout" off the parent blood vessel, where the endothelial cells undergo mitosis and proliferate. The endothelial sprouts merge with each other to form capillary loops, creating the new blood vessel.

Persistent, unregulated angiogenesis occurs in a multiplicity of disease states, tumor metastasis and abnormal growth by endothelial cells and supports the pathological damage seen in these conditions. The diverse pathological disease states in which unregulated angiogenesis is present have been grouped together as angiogenic-dependent, angiogenic-associated, or angiogenic-related diseases. These diseases are a result of abnormal or undesirable cell proliferation, particularly endothelial cell proliferation.

The hypothesis that tumor growth is angiogenesis-dependent was first proposed in 1971 by Judah Folkman (*N. Engl. Jour. Med.* 285:1182 1186, 1971). In its simplest terms the hypothesis proposes that once tumor "take" has occurred, every increase in tumor cell population must be preceded by an increase in new capillaries converging on the tumor. Tumor "take" is currently understood to indicate a prevascular phase of tumor growth in which a population of tumor cells occupying a few cubic millimeters volume and not exceeding a few million cells, survives on existing host microvessels. Expansion of tumor volume beyond this phase requires the

induction of new capillary blood vessels. For example, pulmonary micrometastases in the early prevascular phase in mice would be undetectable except by high power microscopy on histological sections. Further indirect evidence supporting the concept that tumor growth is angiogenesis dependent is found in U.S. Patent Nos. 5,639,725, 5,629,327, 5,792,845, 5,733,876, and 5,854,205, all of which are incorporated herein by reference.

Thus, it is clear that cellular proliferation, particularly endothelial cell proliferation, and most particularly angiogenesis, plays a major role in the metastasis of a cancer. If this abnormal or undesirable proliferation activity could be repressed, inhibited, or eliminated, then the tumor, although present, would not grow. In the disease state, prevention of abnormal or undesirable cellular proliferation and angiogenesis could avert the damage caused by the invasion of the new microvascular system. Therapies directed at control of the cellular proliferative processes could lead to the abrogation or mitigation of these diseases.

What is needed are compositions and methods which can inhibit abnormal or undesirable cellular proliferation, especially the growth of blood vessels into tumors. The compositions should be able to overcome the activity of endogenous growth factors in premetastatic tumors and prevent the formation of the capillaries in the tumors thereby inhibiting the development of disease and the growth of tumors. The compositions should also be able to modulate the formation of capillaries in angiogenic processes, such as wound healing and reproduction. Finally, the compositions and methods for inhibiting cellular proliferation should preferably be non-toxic and produce few side effects.

SUMMARY OF THE INVENTION

Compositions and methods are provided that are effective in inhibiting abnormal or undesirable cell proliferation, particularly endothelial cell proliferation and angiogenesis related to neovascularization and tumor growth. The compositions comprise naturally occurring or synthetic proteins, peptides, or protein fragments containing all, or active fragments of ligands that bind components of the blood coagulation/clotting pathway, optionally provided in a pharmaceutically acceptable carrier.

Representative ligands useful for the present invention comprise proteins or peptides that bind components of both the intrinsic and extrinsic blood clotting pathways such tissue factor (TF), and/or TF cofactor complexes, factor V, factor VIII, factor XII, factor XI, factor X, factor IX, factor VIIa, thrombin, fibrinogen and fibrin.

Preferred ligand compositions of the present invention, include but are not limited to, proteins comprising Kunitz domain proteins, non-Kunitz domain proteins, Kringle-rich proteins, TF cofactors (i.e. factor VIIa, phospholipids, gangliosides), TF antagonists (i.e. antibodies), and any other molecules that bind TF. More particularly, the compositions of the present invention comprise TFPI, protein S, protein Z, protein Z inhibitor, protein C, activated protein C, protein C inhibitor, prothrombin, group II secretory phospholipase A2, complement protein C4b, protease nexin-1, beta2-glycoprotein I, and serpins anticoagulants such as antithrombin and heparin cofactor II.

Preferably, the protein, peptide or protein fragment contains all or an active portion of the above identified proteins. The term "active fragment", as used herein, means a portion of a protein that inhibits abnormal or undesirable cell proliferation, more specifically inhibits endothelial cell proliferation. Also included in the present invention are homologs, peptides, or protein fragments, or

combinations thereof of the above-identified proteins, that inhibit abnormal or undesirable cell proliferation. Most preferably, the protein or peptide comprises TF or factor Xa binding ligand, or an active fragment thereof.

Though not wishing to be bound by the following theory, it is believed that by inhibiting endothelial cell proliferation, the methods and compositions described herein are useful for inhibiting tumor growth and metastasis by blocking tumor vascularization. The methods provided herein for treating diseases and processes mediated by undesired and uncontrolled cell proliferation, such as cancer, involve administering to a human or animal the composition described herein in a dosage sufficient to inhibit cell proliferation, particularly endothelial cell proliferation. The methods are especially useful for treating or repressing the growth of tumors, particularly by inhibiting angiogenesis. Administration of the compositions to a human or animal having prevascularized metastasized tumors is useful for preventing the growth or expansion of such tumors.

Accordingly, it is an object of the present invention to provide methods and compositions for treating diseases and processes that are mediated by abnormal or undesirable cellular proliferation.

It is another object of the present invention to provide methods and compositions for treating or repressing the growth of a cancer.

It is yet another object of the present invention to provide methods and compositions for therapy of cancer that has minimal side effects.

It is another object of the present invention to provide methods and compositions for treating diseases and processes that are mediated by angiogenesis.

Yet another object of the present invention is to provide methods and compositions comprising the use of

proteins, peptides, active fragments and homologs thereof that inhibit endothelial cell proliferation.

Another object of the present invention is to provide methods and compositions for treating diseases and processes that are mediated by angiogenesis by administrating antiangiogenic compounds comprising ligands that bind components of the blood coagulation/clotting pathway.

It is another object of the present invention to provide methods and compositions for treating diseases and processes that are mediated by angiogenesis by administrating antiangiogenic compounds comprising ligands that bind components of the intrinsic and/or extrinsic blood clotting pathways wherein in such components comprise tissue factor (TF), and/or TF cofactor complexes, factor V, factor VIII, factor XII, factor XI, factor X, factor IX, factor VIIa, thrombin, fibrinogen and fibrin.

It is a further object of the present invention to provide methods and compositions for treating diseases and processes that are mediated by angiogenesis by administrating antiangiogenic compounds comprising ligands, wherein the ligands comprise Kunitz domain proteins, non-Kunitz domain proteins, Kringle-rich proteins, TF cofactors, TF antagonists, factor VIIa antagonists or inhibitors and factor Xa antagonists or inhibitors.

It is another object of the present invention to provide methods and compositions for treating diseases and processes that are mediated by angiogenesis comprising administration of antiangiogenic compounds comprising TFPI, protein S, protein Z, protein Z inhibitor, protein C, activated protein C, protein C inhibitor, prothrombin, group II secretory phospholipase A2, complement protein C4b, protease nexin-1, beta2-glycoprotein I, and serpins anticoagulants (such as antithrombin and heparin cofactor II) and inhibitors of factors TF, TF/VIIa, VIIa, Xa.

It is still another object of the present invention to provide antiangiogenic compositions comprising ligands that bind components of the intrinsic and extrinsic blood clotting pathways wherein the compositions further comprise pharmaceutically acceptable carriers.

Yet another object of the present invention is to provide antiangiogenic compositions comprising ligands that bind components of the intrinsic and/or extrinsic blood clotting pathways wherein the compositions further comprise pharmaceutically acceptable carriers that may be administered intramuscularly, intravenously, transdermally, orally, or subcutaneously.

It is yet another object of the present invention to provide compositions and methods for treating diseases and processes that are mediated by angiogenesis including, but not limited to, hemangioma, solid tumors, blood borne tumors, leukemia, metastasis, telangiectasia, psoriasis, scleroderma, pyogenic granuloma, myocardial angiogenesis, Crohn's disease, plaque neovascularization, arteriovenous malformations, corneal diseases, rubeosis, neovascular glaucoma, diabetic retinopathy, retrolental fibroplasia, arthritis, diabetic neovascularization, macular degeneration, wound healing, peptic ulcer, Helicobacter related diseases, fractures, keloids, vasculogenesis, hematopoiesis, ovulation, menstruation, placentation, and cat scratch fever.

These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiment and the appended claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A is a graph showing inhibition of bFGF-induced proliferation of HUVEC cells by TFPI.

Figure 1B is a graph showing increased antiproliferative activity of 0.028μM and 0.139μM TFPI with factor Xa (fXa) in the presence of phospholipids.

Figure 1C is a representation of the results of an SDS-PAGE mobility/Immunoblotting analysis of the mixture TFPI/factor Xa/phospholipids showing that fXa partially cleaves TFPI to fragments with approximate MW 30Kd and 20Kd.

Figure 2A is a graph showing inhibition of bFGF-induced proliferation of HUVEC cells by Heparin Cofactor II (HC-II).

Figure 2B is a graph showing increased antiproliferative activity of HC-II following preincubation of 0.6μM HC-II with fXa in the presence of phospholipids.

Figure 2C is a representation of the results of an SDS-PAGE mobility analysis of the mixture HC-II/factor Xa/phospholipids showing that though there is no significant cleavage of HC-II, cleavage of a limited number of amino acid residues from either N- or C-terminal of the protein is possible.

Figure 3A is a graph showing inhibition of bFGF-induced proliferation of HUVEC cells by Prothrombin (Pro).

Figure 3B is a graph showing increased antiproliferative activity of HC-II following preincubation of 0.55μM Pro with fXa in the presence of phospholipids.

Figure 3C is a representation of the results of an SDS-PAGE mobility analysis of the mixture of Pro/factor Xa/phospholipids revealing complete fragmentation of Pro into three fragments with approximate MW of 40, 30, and 14 Kd.

Figure 4A is a graph showing inhibition of bFGF-induced proliferation of HUVEC cells by Antithrombin III (AT3).

Figure 4B is a graph showing increased antiproliferative activity of AT3 following preincubation of 0.6mM AT3 with fXa in the presence of phospholipids.

Figure 4C is a representation of the results of an SDS-PAGE mobility analysis of the mixture of AT3-II/factor Xa/phospholipids revealing minor cleavage of AT3.

Figure 5 is a graph showing inhibition of bFGF-induced proliferation of HUVEC cells by Protein S.

Figure 6 is a schematic diagram showing the various components of the intrinsic and extrinsic pathways of the blood clotting/coagulation system. (Prior Art)

DETAILED DESCRIPTION

The following description includes the best presently contemplated mode of carrying out the invention. This description is made for the purpose of illustrating the general principles of the invention and should not be taken in a limiting sense. The entire text of the references mentioned herein are hereby incorporated in their entireties by reference, including U.S. Patent Application Serial No. 09/130,273, filed August 6, 1998; and U.S. Patent Application Serial No. 08/796,850, filed February 6, 1997.

Tissue Factor (TF) is a 45 Kd transmembrane glycoprotein that consists of a 219-amino acid extracellular domain, a 23-amino acid transmembrane region, and a 21-amino acid intracellular domain (see Bach, *CRC Crit. Rev. Biochem.* 23:339 (1988). TF binds and allosterically activates factor VIIa (fVIIa) and the complex TF/fVIIa is responsible for thrombin generation via activation of factors IX and X and is the major initiator of blood clotting under physiological conditions. TF has been localized in a variety of tumor and host cells. TF expression is induced by a variety of proinflammatory cytokines, and although it is not synthesized by cells within the vasculature, such as monocytes and endothelial cells, expression is ensured following vascular injury that initiates activation of the blood coagulation cascade. Accordingly it is thought that inflammation, blood coagulation,

and blood vessel formation are interdependent processes and that such processes play an essential role in hematogenous metastasis.

Studies with various experimental metastatic tumor models have shown that, in order to metastasize, tumor cells need to migrate from the site of primary tumor into the blood circulation, travel to a distant target organ, arrest in the microvasculature, extravasate, and grow at the secondary site. In this multistep process, thrombin generation and fibrin formation appear to play important roles. Though not wishing to be bound by the following theory, it is believed that since a majority of the cancer cells that successfully enter the circulation are rapidly eliminated due to blood turbulence, in order to enhance survival, tumor cells tend to aggregate with each other or host cells such as platelets and lymphocytes. They form multicellular emboli (also called thrombi) that are often coated with fibrin. It is thought that these thrombi protect tumor cells against mechanical forces and stabilize the interaction of tumor cells with the microvasculature of the target organ. In addition, thrombin generation on the surface of the tumor cells assists in extravasation by inducing vasodilation of the endothelial cells of the microvasculature, degradation of endothelial cell matrix, and expression of cell-cell adhesion molecules such as intercellular adhesion molecule 1, P-, and E-selectins on the surface of endothelial cells.

The inventors of the present invention have discovered unique compositions and methods for the treatment of diseases and processes that are mediated by, or associated with, abnormal or undesirable cellular proliferation. The compositions comprise isolated naturally occurring or synthetic proteins, peptides, or protein fragments, containing all or an active fragment of ligands that bind components of the intrinsic or extrinsic blood coagulation/clotting pathways, including but not limited to, tissue factor (TF), factor V, factor Va, factor VIIa, factor VIII, factor VIIIa, factor IX,

factor IXa, factor X, factor Xa, factor XI, factor XIa, factor XII, factor XIIa, thrombin, fibrinogen, fibrin,TF cofactor complexes, factor VIIIa complexes, factor Xa complexes, TF/VIIa complexes, factor Xa/Protein Z complexes, factor Xa/factor Va complexes, factor IXa/factor VIII complexes, factor VIIa multicomplexes, factor Xa multicomplexes, and prothrombinase complexes. In addition, the present invention comprises molecules and compounds that effect expression of blood clotting components, and further comprises antibodies and other molecules related to the components. Preferably, the compositions of the present invention comprise naturally occurring or synthetic ligands that bind TF. For delivery to a human or animal, the compositions may optionally comprise a pharmaceutically acceptable carrier or synthetic ligands that bind TF, factors VII and Xa, or their complexes such as prothrombinase complex, complex TF/fVIIa or complex fXa/Protein Z.

Preferably, the ligand compositions of the present invention, include but are not limited to, proteins comprising Kunitz domain proteins, non-Kunitz domain proteins, Kringle-rich proteins, TF cofactors (i.e. factor VIIa, phospholipids, gangliosides), TF antagonists (i.e. antibodies), active fragments thereof, and any other molecules that bind TF. More particularly, the compositions of the present invention may comprise TFPI (binds to factor Xa and complex TF/VIIa), antithrombin (binds to fXa and complex TF/VIIa), prothrombin (binds to factor Xa), heparin cofactor II (binds to thrombin and factor Xa), anticoagulant protein S (binds to factor Xa and factor V), protein C (binds to factor Xa, thrombin), activated protein C (binds to factor V, factor VIII), protein C inhibitor (binds to fXa, thrombin), protein Z (binds to factor Xa), protein Z inhibitor (binds to complex factor Xa/protein Z), tick anticoagulant peptide (binds to factor Xa), protease nexin-1 (binds to factor Xa), beta2-glycoprotein I (binds to factor Xa), complement protein C4b (binds to factor

Xa), and group II secretory phospholipase A2 (binds to factor Xa).

The term "active fragment" is defined herein as the antiproliferative portion of a ligand or molecule necessary for binding a component in the blood coagulation/clotting pathway, preferably tissue factor. The active fragment has the ability to inhibit endothelial cell proliferation by *in vivo* or *in vitro* assays or other known techniques.

As noted above, the compositions of the present invention may be optionally combined with a pharmaceutical carrier. The term "carrier" as used herein comprises delivery mechanisms known to those skilled in the art including, but not limited to, keyhole-limpet hemocyanin (KLH), bovine serum albumin (BSA) and other adjuvants. It is to be understood that the ligand compositions of the present invention can further comprise adjuvants, preservatives, diluents, emulsifiers, stabilizers, and other components that are known and used for pharmaceutical compositions of the prior art. Any adjuvant system known in the art can be used for the compositions of the present invention. Such adjuvants include, but are not limited to, Freund's incomplete adjuvant, Freund's complete adjuvant, polydispersed ß-(1,4) linked acetylated mannan ("Acemannan"), TITERMAX® (polyoxyethylene-polyoxypropylene copolymer adjuvants from CytRx Corporation (Norcross, Georgia), modified lipid adjuvants from Chiron Corporation (Emeryville, California), saponin derivative adjuvants from Aguila Biopharmaceuticals (Worcester, Massachusetts), killed *Bordetella pertussis*, the lipopolysaccharide (LPS) of gram-negative bacteria, large polymeric anions such as dextran sulfate, and inorganic gels such as alum, aluminum hydroxide, or aluminum phosphate, ovalbumin; flagellin; thyroglobulin; serum albumin of any species; gamma globulin of any species; and polymers of D- and/or L- amino acids.

In accordance with the methods of the present invention, the compositions described herein, comprising proteins, peptides, or protein fragments comprising all, or an active fragment of a ligand that binds a blood clotting component, optionally in a pharmaceutically acceptable carrier, is administered to a human or animal exhibiting undesirable cell proliferation in an amount sufficient to inhibit the undesirable cell proliferation, particularly endothelial cell proliferation, angiogenesis or an angiogenesis-related disease, such as cancer.

Definitions

The terms "a", "an" and "the" as used herein are defined to mean one or more and include the plural unless the context is inappropriate.

As used herein the term "blood coagulation factor" is defined to mean an active enzyme and/or its zymogen counterpart. For example, a blood coagulation factor comprises the ligand factor Xa, and also binds to its zymogen counterpart, factor X.

The term "peptides," are chains of amino acids (typically L-amino acids) whose alpha carbons are linked through peptide bonds formed by a condensation reaction between the carboxyl group of the alpha carbon of one amino acid and the amino group of the alpha carbon of another amino acid. The terminal amino acid at one end of the chain (*i.e.*, the amino terminal) has a free amino group, while the terminal amino acid at the other end of the chain (*i.e.*, the carboxy terminal) has a free carboxyl group. As such, the term "amino terminus" (abbreviated N-terminus) refers to the free alpha-amino group on the amino acid at the amino terminal of the peptide, or to the alpha-amino group (imino group when participating in a peptide bond) of an amino acid at any other location within the peptide. Similarly, the term "carboxy terminus" (abbreviated C-terminus) refers to the free carboxyl

group on the amino acid at the carboxy terminus of a peptide, or to the carboxyl group of an amino acid at any other location within the peptide.

Typically, the amino acids making up a peptide are numbered in order, starting at the amino terminal and increasing in the direction toward the carboxy terminal of the peptide. Thus, when one amino acid is said to "follow" another, that amino acid is positioned closer to the carboxy terminal of the peptide than the preceding amino acid.

The term "residue" is used herein to refer to an amino acid (D or L) that is incorporated into a peptide by an amide bond. As such, the amino acid may be a naturally occurring amino acid or, unless otherwise limited, may encompass known analogs of natural amino acids that function in a manner similar to the naturally occurring amino acids (*i.e.*, amino acid mimetics). Moreover, an amide bond mimetic includes peptide backbone modifications well known to those skilled in the art.

The phrase "consisting essentially of" is used herein to exclude any elements that would substantially alter the essential properties of the peptides to which the phrase refers. Thus, the description of a peptide "consisting essentially of . . ." excludes any amino acid substitutions, additions, or deletions that would substantially alter the biological activity of that peptide.

Furthermore, one of skill will recognize that, as mentioned above, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are conservatively modified variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);

2) Aspartic acid (D), Glutamic acid (E);

3) Asparagine (N), Glutamine (Q);

4) Arginine ®, Lysine (K);

5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and

6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany it as found in its native state. Thus, the peptides described herein do not contain materials normally associated with their *in situ* environment. Typically, the isolated, antiproliferative peptides described herein are at least about 80% pure, usually at least about 90%, and preferably at least about 95% as measured by band intensity on a silver stained gel.

Protein purity or homogeneity may be indicated by a number of methods well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualization upon staining. For certain purposes high resolution will be needed and HPLC or a similar means for purification utilized.

When the antiproliferative peptides are relatively short in length (*i.e.*, less than about 50 amino acids), they are often synthesized using standard chemical peptide synthesis techniques.

Solid phase synthesis in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence is a preferred method for the chemical synthesis of the antiproliferative peptides described herein. Techniques for solid phase synthesis are known to those skilled in the art.

Alternatively, the antiproliferative peptides described herein are synthesized using recombinant nucleic acid methodology. Generally, this involves creating a nucleic acid sequence that encodes the peptide, placing the nucleic acid in an expression cassette under the control of a particular promoter, expressing the peptide in a host, isolating the expressed peptide or polypeptide and, if required, renaturing the peptide. Techniques sufficient to guide one of skill through such procedures are found in the literature.

Once expressed, recombinant peptides can be purified according to standard procedures, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like. Substantially pure compositions of about 50 to 95% homogeneity are preferred, and 80 to 95% or greater homogeneity are most preferred for use as therapeutic agents.

One of skill in the art will recognize that after chemical synthesis, biological expression or purification, the antiproliferative peptides may possess a conformation substantially different than the native conformations of the constituent peptides. In this case, it is often necessary to denature and reduce the antiproliferative peptide and then to cause the peptide to re-fold into the preferred conformation. Methods of reducing and denaturing proteins and inducing re-folding are well known to those of skill in the art.

As employed herein, the phrase "biological activity" refers to the functionality, reactivity, and specificity of compounds that are derived from biological systems or those compounds that are reactive to them, or other compounds that mimic the functionality, reactivity, and specificity of these compounds. Examples of suitable biologically active compounds include enzymes, antibodies, antigens and proteins.

The term "bodily fluid," as used herein, includes, but is not limited to, saliva, gingival secretions, cerebrospinal

fluid, gastrointestinal fluid, mucous, urogenital secretions, synovial fluid, blood, serum, plasma, urine, cystic fluid, lymph fluid, ascites, pleural effusion, interstitial fluid, intracellular fluid, ocular fluids, seminal fluid, mammary secretions, and vitreal fluid, and nasal secretions.

Important terms that are used herein are defined as follows. "Cancer" means angiogenesis-dependent cancers and tumors, i.e. tumors that require for their growth (expansion in volume and/or mass) an increase in the number and density of the blood vessels supplying them with blood. "Regression" refers to the reduction of tumor mass and size.

As used herein, the term "angiogenesis" and related terms such as "angiogenic" refer to activities associated with blood vessel growth and development, including, but not limited to, endothelial cell proliferation, endothelial cell migration and capillary tube formation.

As used herein, the term "antiangiogenic" refers to compositions and the like that are capable of inhibiting the formation of blood vessels, including but not limited to inhibiting endothelial cell proliferation, endothelial cell migration and capillary tube formation.

Ligands that Bind Components of the Blood Clotting Pathway

The ligands of the present invention bind components of the intrinsic or extrinsic blood coagulation/clotting pathways, including but not limited to, tissue factor (TF), and/or factor V, factor Va, factor VIIa, factor VIII, factor VIIIa, factor IX, factor IXa, factor X, factor Xa, factor XI, factor XIa, factor XII, factor XIIa, thrombin, fibrinogen, fibrin,TF cofactor complexes, factor VIIIa complexes, factor Xa complexes, TF/VIIa complexes, factor Xa/Protein Z complexes, factor Xa/factor Va complexes, factor IXa/factor VIII complexes, factor VIIa multicomplexes, factor Xa multicomplexes, and prothrombinase complexes. Most preferably the ligands of the

present invention bind TF, fVIIa, fXa, and/or their multimolecular complexes such as TF/fVIIa complex, prothrombinase complex, fXa/protein Z complex.

Although blood clotting is usually associated with wound healing, it also accompanies a variety of other pathological conditions. The inventors of the present invention have discovered that whereas normal endothelial cells do not synthesize TF, tumor vascular endothelial cells express TF at the onset of tumor angiogenesis. Though not wishing to be bound by the following theory, because TFPI has been shown to inhibit endothelial cell growth (see U.S. Patent Application Serial No. 09/130,273, filed August 6, 1998), it is conceivable that, under conditions that promote blood vessel formation, TF mediates the antiproliferative action of TFPI. Since TFPI requires binding to fXa, it is possible that fXa also participates in the transduction of TFPI's antimitotic signal. Moreover, since TF requires fVIIa in order to bind to TFPI/fXIIa, it is also feasible that fVIIa is part of a multimolecular complex that promotes inhibition of angiogenesis. Accordingly, the methods and compositions of the present invention comprise all ligands that bind TF, fVIIa, TFPI/fXIIa, and effect the blood coagulation/clotting pathway by inhibiting angiogenesis.

In addition, the compositions of the present invention further comprise molecules and compounds that effect expression of blood clotting components, and further comprises antibodies and other molecules related to the components. Preferably, the compositions of the present invention include, but are not limited to, proteins comprising Kunitz domain proteins, non-Kunitz domain proteins, Kringle-rich proteins, TF cofactors (i.e. factor VIIa, phospholipids, gangliosides), TF antagonists (i.e. antibodies), and any other molecules that bind TF. More particularly, the compositions of the present invention comprise TFPI, protein S, protein Z, protein Z inhibitor, protein C, activated protein C, protein C inhibitor, prothrombin, group II secretory phospholipase A2,

complement protein C4b, protease nexin-1, beta2-glycoprotein I, and serpin anticoagulants (such as antithrombin and particularly heparin cofactor II), and inhibitors of factors TF, TF/VIIa, VIIa, Xa. Most preferably, the compositions of the present invention comprise naturally occurring or synthetic ligands that bind TF.

The ligands of the present invention may be isolated from body fluids including, but not limited to, serum, urine, and ascites, or may be synthesized by chemical or biological methods, such as cell culture, recombinant gene expression, and peptide synthesis. Recombinant techniques include gene amplification from DNA sources using the polymerase chain reaction (PCR), and gene amplification from RNA sources using reverse transcriptase/PCR. LDLR ligands are extracted from body fluids by known protein extraction methods, particularly the method described by Novotny, W.F., *et al., J. Biol. Chem.* 264:18832-18837 (1989).

The inventors of the present invention surprisingly demonstrated the antiproliferative, or more specifically, antiangiogenic properties of the claimed compositions and methods by showing that molecules that bind to components of the blood coagulation/clotting pathway such as TF/factor VIIa complex (i.e., TFPI), or factor Xa (i.e., protein S), inhibit endothelial cell proliferation (see Examples). In addition, the data generated by the inventors demonstrate that binding of a ligand to factor Xa enhances the antiproliferative activity of the ligand (see Figures 1B, 2B, 3B, and 4B). Though not wishing to be bound by the following theory, it is believed that in some cases, proteolytic cleavage of the ligand by factor Xa may account for the augmentation of the antiproliferative activity. Indeed, antiangiogenic proteins are often released after a specific cleavage of the parent molecules that possess none or little antiangiogenic activity. For example, plasminogen is cleaved to release ANGIOSTATIN® protein, collagen XVIII is cleaved to

release ENDOSTATIN® protein, prolactin is cleaved to release a 16 Kd fragment with potent antiangiogenic properties, while platelet factor-4, a weak inhibitor of angiogenesis, can be cleaved to a fragment that is 50-times more potent than platelet factor-4. Accordingly, as demonstrated by Dr. Folkman, inhibitors of angiogenesis are sometimes "hidden" away as parts of large, abundant but inert molecules that are cleaved to release the antiangiogenic fragments when needed (Hanahan, D., and Folkman, J. Cell 86, 353 (1996)). Blood coagulation often precedes or accompanies formation of new blood vessels (in cases like wound healing or tumor growth), and the inventors of the present invention discovered that blood clotting proteins are cleaved to provide the inhibitors necessary for the regulation of the angiogenic process. Since blood coagulation involves several proteases, one or more of these enzymes (such as factors VIIa and Xa) may participate in the cleavage. For example, factor Xa can cleave prothrombin to release an antiangiogenic fragment.

Accordingly, the present invention further comprises antiangiogenic fragments released by proteolytic cleavage of blood clotting pathway components (i.e., fXa-binding or fXa-mediated proteolytic cleavage, therefore ligands to fXa or proteins that are modified by fXa or another blood coagulation protease such as thrombin are inhibitors of angiogenesis).

Peptides or Protein Fragments

Peptides or protein fragments comprising ligands that bind components of the blood coagulation/clotting pathway, such as TF binding ligands, can be produced from the proteins described above and tested for antiproliferative or antiangiogenic activity using techniques and methods known to those skilled in the art. For example, full length recombinant TFPI (rTFPI) can be produced using the Baculovirus gene expression system. Full length proteins can be cleaved into

individual domains or digested using various methods such as, for example, the method described by Enjyoji *et al.* (*Biochemistry* 34:5725-5735 (1995)). In accordance with the method of Enjyoji *et al.*, rTFPI is treated with a digestion enzyme, human neutrophil elastase, and the digest purified using a heparin column. Human neutrophil elastase cleaves TFPI at Leu^{89} into two fragments: one containing Kunitz-1 and the other containing Kunitz-2 and Kunitz-3. To produce additional fragments, the fragment containing Kunitz-2 and Kunitz-3 (Kunitz-2/Kunitz-3) is preferably treated with a digestion compound, hydroxylamine, according to the method of Balian *et al.* (*Biochemistry* 11:3798-3806 (1972)), and the digest purified using a heparin column. Hydroxylamine cleaves the fragment containing Kunitz-2 and Kunitz-3 into two fragments: one containing Kunitz-3 and the other containing the Kunitz-2 domain.

Alternatively, fragments are prepared by digesting the entire protein, or large fragments thereof exhibiting anti-proliferative activity, to remove one amino acid at a time. Each progressively shorter fragment is then tested for anti-proliferative activity. Similarly, fragments of various lengths may be synthesized and tested for anti-proliferative activity. By increasing or decreasing the length of a fragment, one skilled in the art may determine the exact number, identity, and sequence of amino acids within the protein that are required for anti-proliferative activity using routine digestion, synthesis, and screening procedures known to those skilled in the art.

Anti-proliferative activity is evaluated *in situ* by testing the ability of the fragments to inhibit the proliferation of new blood vessel cells, referred to herein as the inhibition of angiogenesis. A suitable assay is the chick embryo chorioallantoic membrane (CAM) assay described by Crum *et al.*, *Science* 230:1375 (1985) and described in U.S. Patent No. 5,001,116, which is incorporated by reference herein. The

CAM assay is briefly described as follows. Fertilized chick embryos are removed from their shell on day 3 or 4, and a methylcellulose disc containing the fragment of interest is implanted on the chorioallantoic membrane. The embryos are examined 48 hours later and, if a clear avascular zone appears around the methylcellulose disc, the diameter of that zone is measured. The larger the diameter of the zone, the greater the anti-angiogenic activity. Another suitable assay is the HUVEC assay as described in Example 1.

The active fragment is preferably a fragment containing that portion of the ligand that is necessary for binding TF. In particular, ligands having either Kunitz or non-Kunitz domains, or Kringle-rich proteins are preferred. As discussed above, one of skill in the art will recognize that, individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are conservatively modified variations where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Accordingly, also included in the present invention are peptides having conservatively modified variations in comparison to the claimed peptides, wherein the chemical reactivity of the peptide is not significantly different from that of the claimed peptide.

Formulations

The naturally occurring or synthetic protein, peptide, or protein fragment, containing all or an active fragment of a ligand that may bind to a component of the blood clotting pathway can be prepared in a physiologically acceptable formulation, such as in a pharmaceutically acceptable carrier, using known techniques. For example, the protein, peptide or protein fragment is combined with a

pharmaceutically acceptable excipient to form a therapeutic composition.

Alternatively, the gene for the protein, peptide, or protein fragment, containing all or an active fragment of a desired ligand, may be delivered in a vector for continuous administration using gene therapy techniques. The vector may be administered in a vehicle having specificity for a target site, such as a tumor.

The composition may be in the form of a solid, liquid or aerosol. Examples of solid compositions include pills, creams, and implantable dosage units. Pills may be administered orally. Therapeutic creams may be administered topically. Implantable dosage units may be administered locally, for example, at a tumor site, or may be implanted for systematic release of the therapeutic composition, for example, subcutaneously. Examples of liquid compositions include formulations adapted for injection subcutaneously, intravenously, intra-arterially, and formulations for topical and intraocular administration. Examples of aerosol formulations include inhaler formulations for administration to the lungs.

The composition may be administered by standard routes of administration. In general, the composition may be administered by topical, oral, rectal, nasal or parenteral (for example, intravenous, subcutaneous, or intermuscular) routes. In addition, the composition may be incorporated into sustained release matrices such as biodegradable polymers, the polymers being implanted in the vicinity of where delivery is desired, for example, at the site of a tumor. The method includes administration of a single dose, administration of repeated doses at predetermined time intervals, and sustained administration for a predetermined period of time.

A sustained release matrix, as used herein, is a matrix made of materials, usually polymers which are degradable by enzymatic or acid/base hydrolysis or by

dissolution. Once inserted into the body, the matrix is acted upon by enzymes and body fluids. The sustained release matrix desirably is chosen by biocompatible materials such as liposomes, polylactides (polylactide acid), polyglycolide (polymer of glycolic acid), polylactide co-glycolide (copolymers of lactic acid and glycolic acid), polyanhydrides, poly(ortho)esters, polypeptides, hyaluronic acid, collagen, chondroitin sulfate, carboxylic acids, fatty acids, phospholipids, polysaccharides, nucleic acids, polyamino acids, amino acids such phenylalanine, tyrosine, isoleucine, polynucleotides, polyvinyl propylene, polyvinylpyrrolidone and silicone. A preferred biodegradable matrix is a matrix of one of either polylactide, polyglycolide, or polylactide co-glycolide (co-polymers of lactic acid and glycolic acid).

The dosage of the composition will depend on the condition being treated, the particular composition used, and other clinical factors such as weight and condition of the patient, and the route of administration.

Further, the term "effective amount" refers to the amount of the composition which, when administered to a human or animal, inhibits undesirable cell proliferation, particularly endothelial cell proliferation, causing a reduction in cancer or inhibition in the spread and proliferation of cancer. The effective amount is readily determined by one of skill in the art following routine procedures. For example, antiproliferative compositions of the present invention may be administered parenterally or orally in a range of approximately 1.0 μg to 1.0 mg per patient, though this range is not intended to be limiting. The actual amount of antiproliferative composition required to elicit an appropriate response will vary for each individual patient depending on the potency of the composition administered and on the response of the individual. Consequently, the specific amount administered to an individual will be determined by routine

experimentation and based upon the training and experience of one skilled in the art.

The composition may be administered in combination with other compositions and procedures for the treatment of diseases. For example, unwanted cell proliferation may be treated conventionally with surgery, radiation or chemotherapy in combination with the administration of the composition, and additional doses of the composition may be subsequently administered to the patient to stabilize and inhibit the growth of any residual unwanted cell proliferation.

Antibodies of Ligands that Bind Blood Clotting Components

The present invention further comprises antibodies of ligands that bind blood coagulation/clotting components that may be used for diagnostic as well as therapeutic purposes. The antibodies provided herein are monoclonal or polyclonal antibodies having binding specificity for desired ligands. The preferred antibodies are monoclonal antibodies, due to their higher specificity for the ligands. The antibodies exhibit minimal or no crossreactivity with other proteins or peptides. Preferably, the antibodies are specific for ligands comprising Kunitz domain proteins, non-Kunitz domain proteins, Kringle-rich proteins, TF cofactors (i.e. factor V, factor VIIa, factor Xa, phospholipids, gangliosides), TF antagonists (i.e. antibodies), and any other molecules that bind TF including but not limited to sPLA2, APC, anticoagulant protein S, tick anticoagulant peptide, TFPI, thrombin and antithrombin. Most preferably the antibodies are specific for TF ligands.

Monoclonal antibodies are prepared by immunizing an animal, such as a mouse or rabbit, with a whole or immunogenic portion of a desired ligand, such as antithrombin III. Spleen cells are harvested from the immunized animals and hybridomas generated by fusing

sensitized spleen cells with a myeloma cell line, such as murine SP2/O myeloma cells (ATCC, Manassas, VA). The cells are induced to fuse by the addition of polyethylene glycol. Hybridomas are chemically selected by plating the cells in a selection medium containing hypoxanthine, aminopterin and thymidine (HAT).

Hybridomas are subsequently screened for the ability to produce monoclonal antibodies against ligands. Hybridomas producing antibodies that bind to the ligands are cloned, expanded and stored frozen for future production. The preferred hybridoma produces a monoclonal antibody having the IgG isotype, more preferably the IgG1 isotype.

The polyclonal antibodies are prepared by immunizing animals, such as mice or rabbits with a ligand such as antithrombin as described above. Blood sera is subsequently collected from the animals, and antibodies in the sera screened for binding reactivity against the ligand, preferably the antigens that are reactive with the monoclonal antibody described above.

Either the monoclonal antibodies or the polyclonal antibodies, or both may be labeled directly with a detectable label for identification and quantitation of ligands in a biological as described below. Labels for use in immunoassays are generally known to those skilled in the art and include enzymes, radioisotopes, and fluorescent, luminescent and chromogenic substances including colored particles, such as colloidal gold and latex beads. The antibodies may also be bound to a solid phase to facilitate separation of antibody-antigen complexes from non-reacted components in an immunoassay. Exemplary solid phase substances include, but are not limited to, microtiter plates, test tubes, magnetic, plastic or glass beads and slides. Methods for coupling antibodies to solid phases are well known to those skilled in the art.

Alternatively, the antibodies may be labeled indirectly by reaction with labeled substances that have an affinity for immunoglobulin, such as protein A or G or second antibodies. The antibodies may be conjugated with a second substance and detected with a labeled third substance having an affinity for the second substance conjugated to the antibody. For example, the antibodies may be conjugated to biotin and the antibody-biotin conjugate detected using labeled avidin or streptavidin. Similarly, the antibodies may be conjugated to a hapten and the antibody-hapten conjugate detected using labeled anti-hapten antibody. These and other methods of labeling antibodies and assay conjugates are well known to those skilled in the art.

Sensitive immunoassays employing one or more of the antibodies described above are provided by the present invention. The immunoassays are useful for detecting the presence or amount of ligands in a variety of samples, particularly biological samples, such as human or animal biological fluids. The samples may be obtained from any source in which the ligands may exist. For example, the sample may include, but is not limited to, blood, saliva, semen, tears, and urine.

The antibody-antigen complexes formed in the immunoassays of the present invention are detected using immunoassay methods known to those skilled in the art, including sandwich immunoassays and competitive immunoassays. The antibody-antigen complexes are exposed to antibodies similar to those used to capture the antigen, but which have been labeled with a detectable label. Suitable labels include: chemiluminescent labels, such as horseradish peroxidase; electrochemiluminescent labels, such as ruthenium and aequorin; bioluminescent labels, such as luciferase; fluorescent labels such as FITC; and enzymatic labels such as alkaline phosphatase, ß-galactosidase, and horseradish peroxidase.

The labeled complex is then detected using a detection technique or instrument specific for detection of the label employed. Soluble antigen or antigens may also be incubated with magnetic beads coated with non-specific antibodies in an identical assay format to determine the background values of samples analyzed in the assay.

Pharmaceutical Compositions

The compositions of the present invention are useful in therapeutic and prophylactic applications for the treatment of diseases mediated by undesirable cell proliferation such as cancer.

As such, the present invention provides pharmaceutical compositions wherein the compositions generally comprise one more ligands, or active fragments thereof that bind components of the blood clotting pathway together with a pharmaceutically acceptable carrier. Such compositions are suitable for use in a variety of drug delivery systems. Suitable formulations for use in the present invention are found in *Remington's Pharmaceutical Sciences,* Mack Publishing Company, Philadelphia, PA, 17th ed. (1985), which is incorporated herein by reference. For a brief review of methods for drug delivery, see, Langer, *Science* 249:1 527-1533 (1990), which is incorporated herein by reference.

The pharmaceutical compositions of the invention are intended for parenteral, topical, oral or local administration. Preferably, the pharmaceutical compositions are administered parenterally, *e.g.*, intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration that comprise a solution of the agents described above dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers may be used, *e.g.*, water, buffered water, 0.4% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be

sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc.

For solid compositions, conventional nontoxic solid carriers may be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient and more preferably at a concentration of 25%-75%.

For aerosol administration, the polypeptides are preferably supplied in finely divided form along with a surfactant and propellant. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. A carrier can also be included, as desired, as with, *e.g.*, lecithin for intranasal delivery.

The amount administered to the patient will vary depending upon what is being administered, the state of the patient and the manner of administration. In therapeutic

applications, compositions are administered to a patient already suffering from a disease mediated by undesirable cell proliferation, in an amount sufficient to inhibit cell proliferation, or at least partially arrest the symptoms of the disease and its complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on the severity of the disease, the particular composition, and the weight and general state of the patient. Generally, the dose will be in the range of about 0.5 mg to about 5 mg per day, preferably about 100 mg per day, for a 70 kg patient.

In a preferred embodiment, the ligands, or active fragments thereof that bind components of the blood clotting pathway are covalently attached (conjugated) to a carrier protein as described above. Useful carrier proteins include, but are not limited to, thyroglobulin, albumins such as human serum albumin, tetanus toxoid, polyamino acids such as poly(D-lysine:D-glutamic acid), influenza. The compositions can also contain a physiologically tolerable (acceptable) diluent such as water, phosphate buffered saline, or saline, and further typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or alum are materials well known in the art.

In addition, DNA or RNA encoding the ligands, or active fragments thereof, that bind components of the blood clotting pathway may be introduced into patients to obtain an immune response to the immunogenic peptides which the nucleic acid encodes. *See*, Wolff, *et al.*, *Science* 247: 1465-1468 (1990) which describes the use of nucleic acids to produce expression of the ligands which the nucleic acids encode, the teachings of which are incorporated herein by reference.

Diseases and Conditions to be Treated

The methods and compositions described herein are useful for treating human and animal diseases and processes mediated by abnormal or undesirable cellular proliferation, particularly abnormal or undesirable endothelial cell proliferation, including, but not limited to, hemangioma, solid tumors, leukemia, metastasis, telangiectasia psoriasis scleroderma, pyogenic granuloma, myocardial angiogenesis, plaque neovascularization, coronary collaterals, ischemic limb angiogenesis, corneal diseases, rubeosis, neovascular glaucoma, diabetic retinopathy, retrolental fibroplasia, arthritis, diabetic neovascularization, macular degeneration, wound healing, peptic ulcer, fractures, keloids, vasculogenesis, hematopoiesis, ovulation, menstruation, and placentation. The method and composition are particularly useful for treating angiogenesis-related disorders and diseases by inhibiting angiogenesis.

The methods and compositions described herein are particularly useful for treating cancer, arthritis, macular degeneration, and diabetic retinopathy. Administration of the compositions to a human or animal having prevascularized metastasized tumors is useful for preventing the growth or expansion of such tumors.

The compositions and methods are further illustrated by the following non-limiting examples, which are not to be construed in any way as imposing limitations upon the scope thereof. On the contrary, it is to be clearly understood that resort may be had to various other embodiments, modifications, and equivalents thereof which, after reading the description herein, may suggest themselves to those skilled in the art without departing from the spirit of the present invention and/or the scope of the appended claims.

EXAMPLE 1

Inhibition of Human Umbilical Vascular Endothelial Cell Proliferation by TFPI and Factor Xa

Human Umbilical Vascular Endothelial Cell Proliferation Assay

Human umbilical vascular endothelial cells (HUVECs) and their media (EGM and EBM) were purchased from Clonetics (San Diego, CA).

HUVECs were routinely cultured to confluency in EGM. The cells were trypsinized and plated in a 96-well plate at 5,000 cells per 100 µl EBM supplemented with 2% serum and antibiotics. The cells were allowed to adhere to the plate for at least 2 hrs. Then, bFGF at 10 ng/ml and various concentrations of an antiangiogenic agent were added to the wells. The cells were cultured for 48 hrs at 37°C in a 5% CO_2 atmosphere. Cell proliferation was determined using a uridine incorporation method (Boehringer Mannheim Corporation, Indianapolis, IN).

As shown in Figure 1A, TFPI inhibits bFGF-induced proliferation of HUVEC cells. Preincubation of 0.028µM and 0.139µM TFPI with factor Xa (fXa) in the presence of phospholipids led to an increased antiproliferative activity of these two concentrations of TFPI (Figure 1B). SDS-PAGE mobility/Immunoblotting of the mixture TFPI/factor Xa/phospholipids revealed that fXa partially cleaved TFPI to fragments with approximate MW 30Kd and 20Kd (Figure 1C).

Based on the findings of this experiment, it is likely that complexing with factor Xa and/or fragmentation of TFPI may enhance the antiangiogenic activity of TFPI.

EXAMPLE 2

Inhibition of Human Umbilical Vascular Endothelial Cell Proliferation by Heparin Cofactor II

Using a HUVEC assay as described above in Example 1, it was demonstrated that Heparin Cofactor II (HC-II) inhibits bFGF-induced proliferation of HUVEC cells (Figure 2A). Preincubation of 0.6μM HC-II with fXa in the presence of phospholipids led to an increased antiproliferative activity (Figure 2B). SDS-PAGE mobility of the mixture HC-II/factor Xa/phospholipids did not reveal any significant cleavage of HC-II, although cleavage of a limited number of amino acid residues from either N- or C-terminal of the protein is possible, especially since there is a slight down-shift of the HC-II band after incubation with fXa (Figure 2C).

Based on the findings of this experiment, it is likely that complexing HC-II with factor Xa and/or proteolytic cleavage of limited number of residues of HC-II enhances the antiangiogenic activity of HC-II.

EXAMPLE 3

Inhibition of Human Umbilical Vascular Endothelial Cell Proliferation by Prothrombin

Using a HUVEC assay as described above in Example 1, it was demonstrated that Prothrombin (Pro) inhibits bFGF-induced proliferation of HUVEC cells (Figure 3A). Preincubation of 0.55μM Pro with fXa in the presence of phospholipids led to an increased antiproliferative activity (Figure 2B). SDS-PAGE mobility of the mixture Pro/factor Xa/phospholipids revealed complete fragmentation of Pro into three fragments with approximate MW of 40, 30, and 14 Kd (Figure 3C).

Based on the findings of this experiment, it is likely that complexing prothrombin with factor Xa enhances the antiangiogenic activity of prothrombin.

EXAMPLE 4

Inhibition of Human Umbilical Vascular Endothelial Cell Proliferation by Antithrombin III

Using a HUVEC assay as described above in Example 1, it was demonstrated that Antithrombin III (AT3) inhibits bFGF-induced proliferation of HUVEC cells (Figure 4A). Preincubation of 0.6mM AT3 with fXa in the presence of phospholipids led to an increased antiproliferative activity (Figure 4B). SDS-PAGE mobility/Immunoblotting of the mixture AT3-II/factor Xa/phospholipids revealed minor cleavage of AT3 (Figure 4C).

Based on the findings of this experiment, it is likely that complexing with factor Xa and/or proteolytic cleavage of limited number of residues of AT3 may enhance the antiangiogenic activity of AT3.

EXAMPLE 5

Inhibition of Human Umbilical Vascular Endothelial Cell Proliferation by Antithrombin III

Using a HUVEC assay as described above in Example 1, it was demonstrated that Protein S inhibits bFGF-induced proliferation of HUVEC cells (Figure 5).

CLAIMS

We claim:

1. A method of treating a human or animal having undesirable cell proliferation comprising,

administering to the human or animal an effective amount of a composition comprising an isolated protein or peptide, or antiproliferative fragment thereof, wherein the protein or peptide comprises a ligand that is capable of binding to a component of the blood clotting pathway,

wherein the effective amount is sufficient to inhibit the undesirable cell proliferation.

2. The method of Claim 1, wherein component of the blood clotting pathway comprises tissue factor, factor V, factor Va, factor VIIa, factor VIII, factor VIIIa, factor IX, factor IXa, factor X, factor Xa, factor XI, factor XIa, factor XII, factor XIIa, thrombin, fibrinogen, fibrin,TF cofactor complexes, factor VIIIa complexes, factor Xa complexes, TF/VIIa complexes, factor Xa/Protein Z complexes, factor Xa/factor Va complexes, factor IXa/factor VIII complexes, factor VIIa multicomplexes, factor Xa multicomplexes, and prothrombinase complexes.

3. The method of Claim 1, wherein isolated protein or peptide comprises a ligand selected from the group consisting of Kunitz domain proteins, non-Kunitz domain proteins, Kringle-rich proteins, TF cofactors, TF antagonists TF antibodies and active fragments thereof.

4. The method of Claim 3, wherein the TF cofactors comprise factor VIIa, phospholipids, and gangliosides.

5. The method of Claim 1, wherein the isolated protein or peptide comprises a ligand selected from the group

consisting of TFPI, antithrombin III, prothrombin, heparin cofactor II, anticoagulant protein, protein C, activated protein C, protein C inhibitor, protein S, protein Z, protein Z inhibitor, tick anticoagulant peptide, protease nexin-1, beta2-glycoprotein I, complement protein C4b, and group II secretory phospholipase A2.

6. The method of Claim 5, wherein the ligand is selected from the group consisting of thrombin, prothrombin, and antithrombin III.

7. The method of Claim 1, wherein the composition further comprises a pharmaceutically acceptable excipient, carrier or sustained-release matrix.

8. The method of Claim 1, wherein the undesirable cell proliferation is undesirable endothelial cell proliferation.

9. The method of Claim 8 wherein the inhibition of endothelial cell proliferation inhibits neovascularization.

10. The method of Claim 1, wherein the undesirable cell proliferation is an angiogenesis-related disease.

11. The method of Claim 10, wherein the angiogenic-related disease is a disease selected from the group consisting of cancer, arthritis, macular degeneration, and diabetic retinopathy.

12. A method of treating undesired angiogenesis in a human or animal comprising the steps of administering to the human or animal with the undesired angiogenesis a composition comprising an effective amount of an angiogenesis-inhibiting compound comprising a ligand that is capable of binding to a component of the blood clotting pathway.

13. The method of Claim 12, wherein the wherein component of the blood clotting pathway comprises tissue factor, factor V, factor Va, factor VIIa, factor VIII, factor VIIIa, factor IX, factor IXa, factor X, factor Xa, factor XI, factor XIa, factor XII, factor XIIa, thrombin, fibrinogen, fibrin,TF cofactor complexes, factor VIIIa complexes, factor Xa complexes, TF/VIIa complexes, factor Xa/Protein Z complexes, factor Xa/factor Va complexes, factor IXa/factor VIII complexes, factor VIIa multicomplexes, factor Xa multicomplexes, and prothrombinase complexes.

14. The method of Claim 12, wherein isolated protein or peptide comprises a ligand selected from the group consisting of Kunitz domain proteins, non-Kunitz domain proteins, Kringle-rich proteins, TF cofactors, TF antagonists TF antibodies and active fragments thereof.

15. The method of Claim 14, wherein the isolated protein or peptide comprises a ligand selected from the group consisting of TFPI, antithrombin III, prothrombin, heparin cofactor II, anticoagulant protein, protein C, activated protein C, protein C inhibitor, protein S, protein Z, protein Z inhibitor, tick anticoagulant peptide, protease nexin-1, beta2-glycoprotein I, complement protein C4b, and group II secretory phospholipase A2.

16. The method of Claim 14, wherein the undesired angiogenesis is related to an angiogenic-related disease comprising cancer, arthritis, macular degeneration, and diabetic retinopathy.

17. A composition for treating undesired angiogenesis in a human or animal comprising an effective amount of an angiogenesis-inhibiting compound wherein the compound comprises ligands that are capable of binding to a component of the blood clotting pathway.

18. The composition of Claim 17, the wherein component of the blood clotting pathway comprises tissue factor, factor V, factor Va, factor VIIa, factor VIII, factor VIIIa, factor IX, factor IXa, factor X, factor Xa, factor XI, factor XIa, factor XII, factor XIIa, thrombin, fibrinogen, fibrin,TF cofactor complexes, factor VIIIa complexes, factor Xa complexes, TF/VIIa complexes, factor Xa/Protein Z complexes, factor Xa/factor Va complexes, factor IXa/factor VIII complexes, factor VIIa multicomplexes, factor Xa multicomplexes, and prothrombinase complexes.

19. The composition of Claim 17, wherein the is ligand selected from the group consisting of Kunitz domain proteins, non-Kunitz domain proteins, Kringle-rich proteins, TF cofactors, TF antagonists TF antibodies and active fragments thereof

20. The composition of Claim 17, wherein the ligand is selected from the group consisting of antithrombin III, tissue factor pathway inhibitor and active fragments thereof.

FIGURE 1A

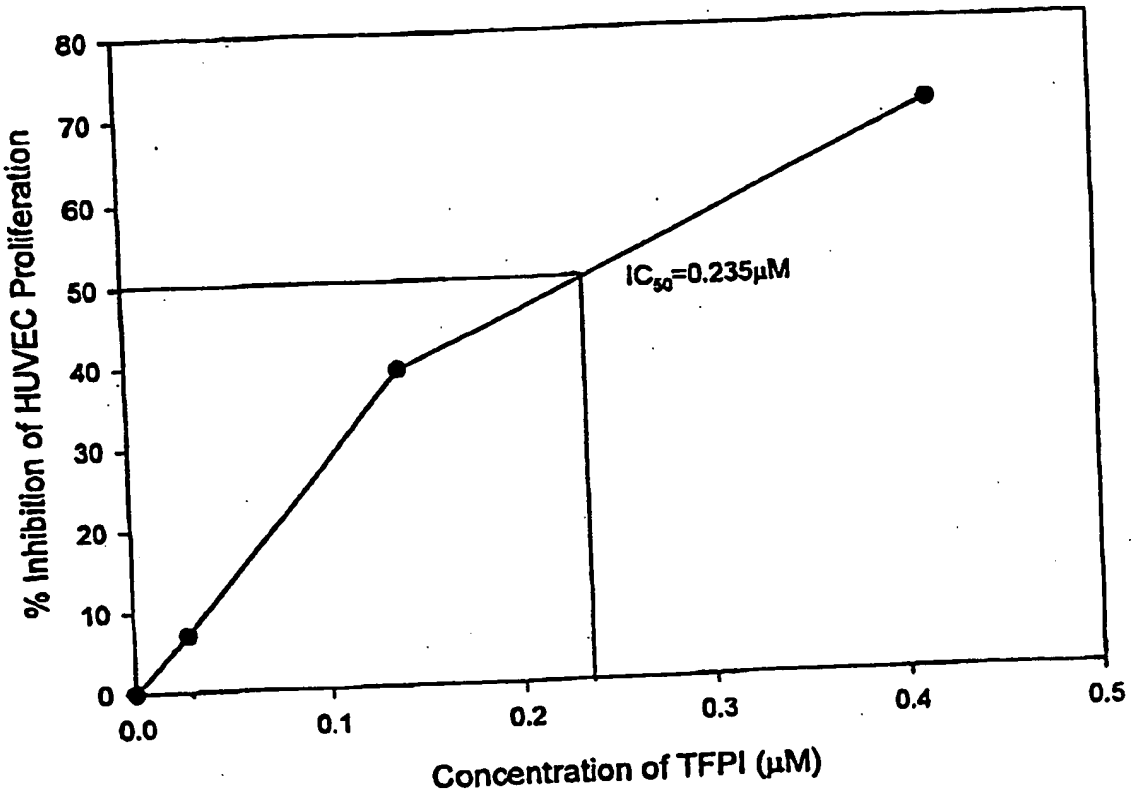
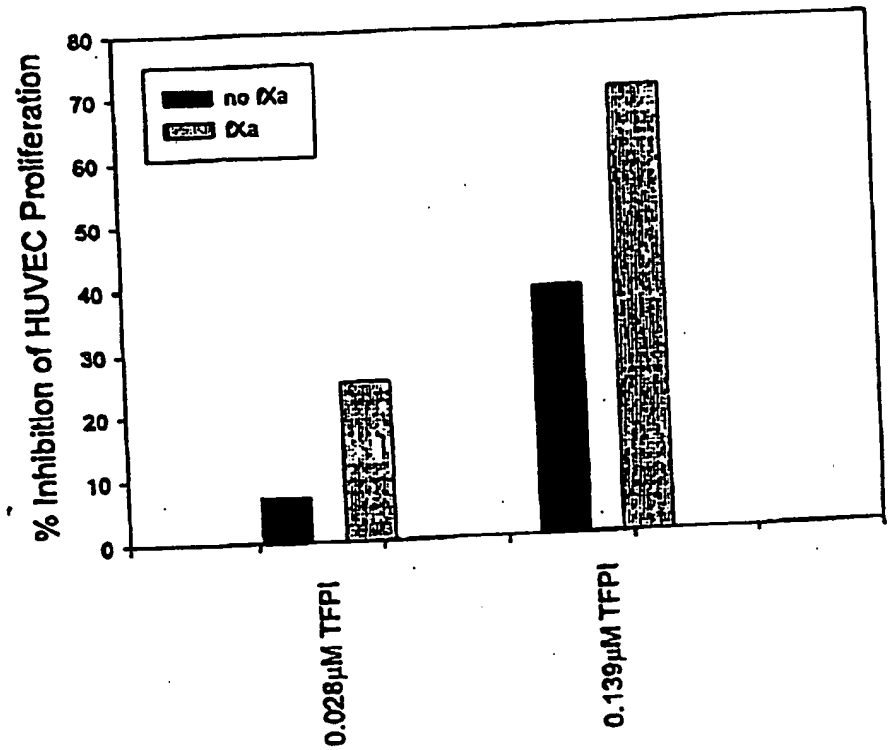


FIGURE 1B



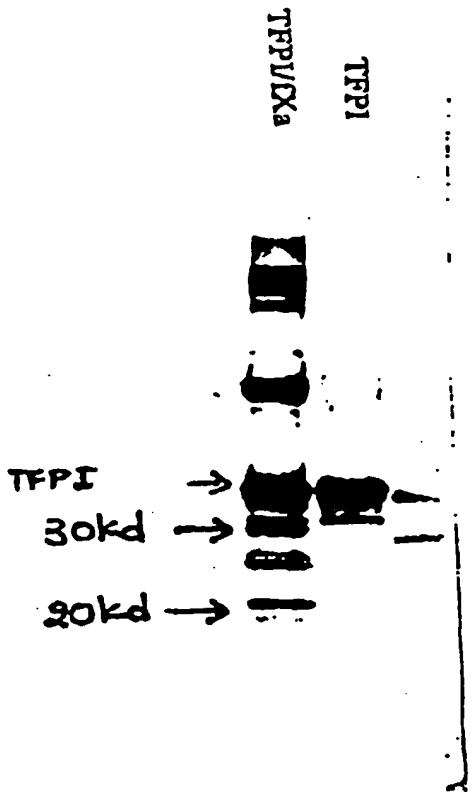


FIGURE 1C

FIGURE 2A

3/10

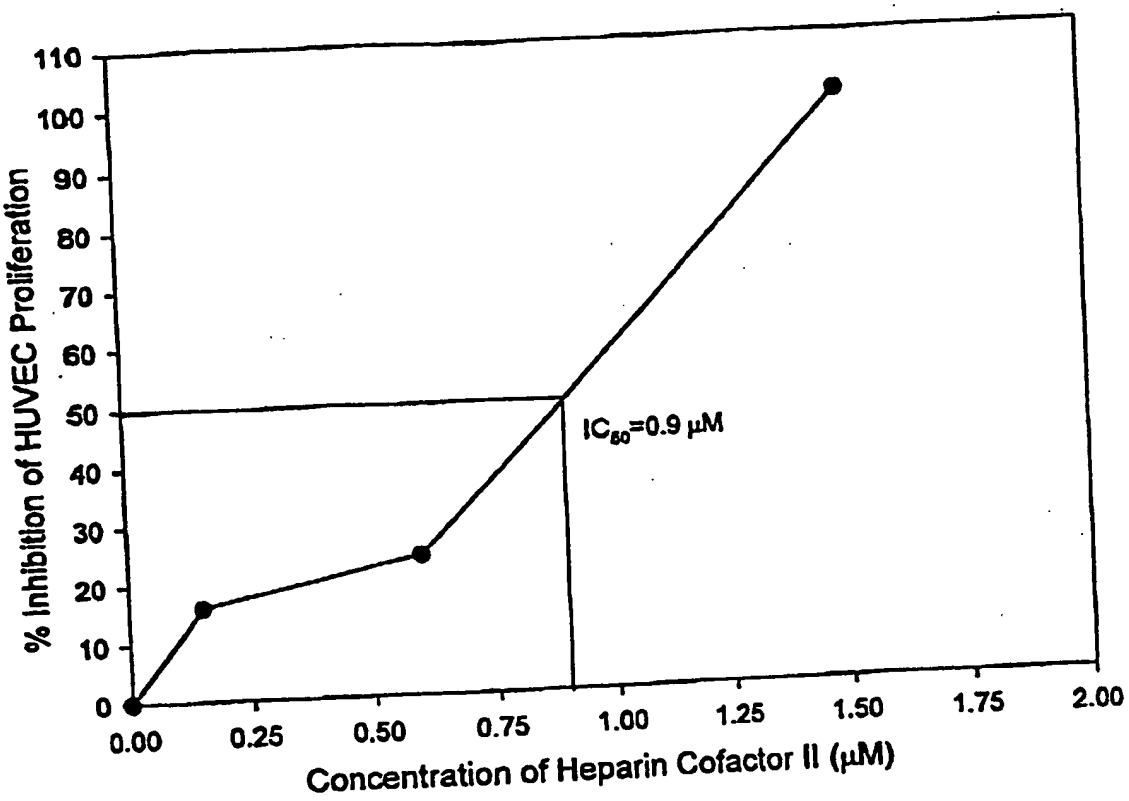
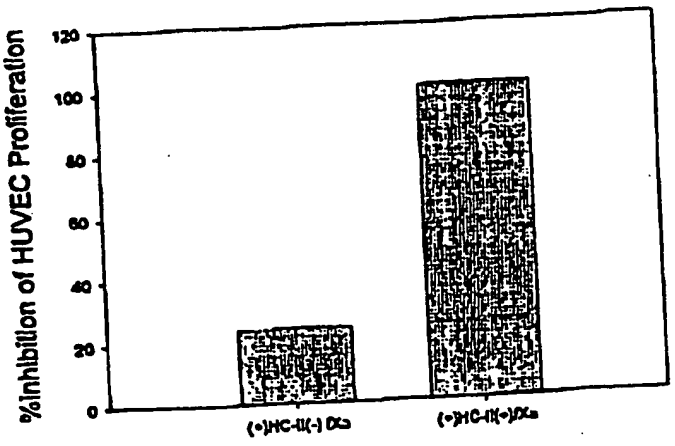


FIGURE 2B



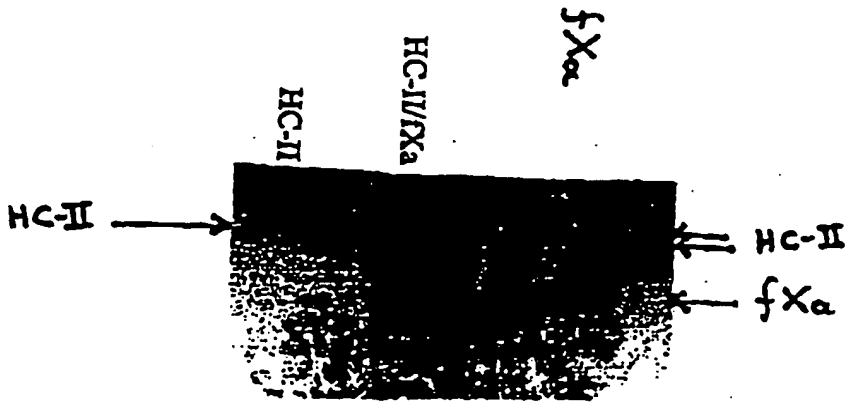


FIGURE 2C

FIGURE 3A

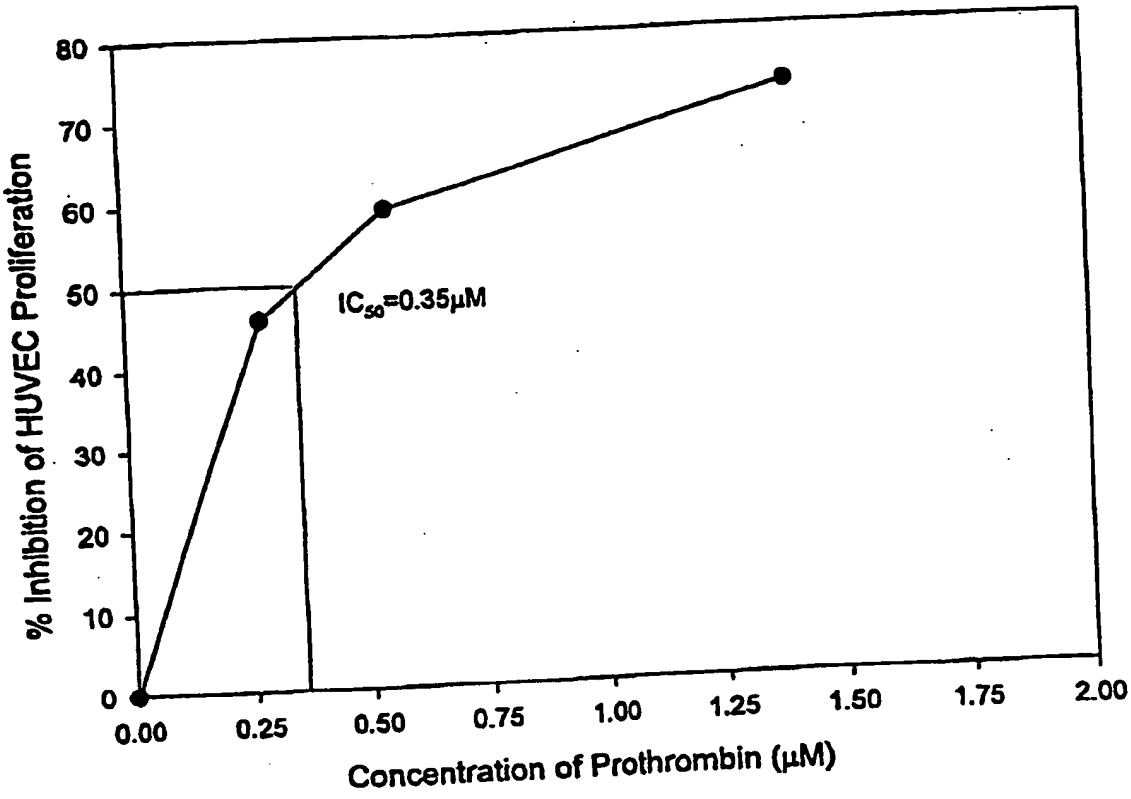
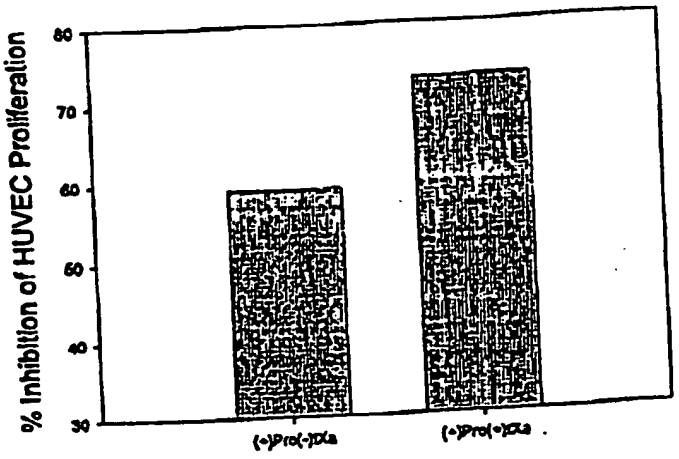


FIGURE 3B



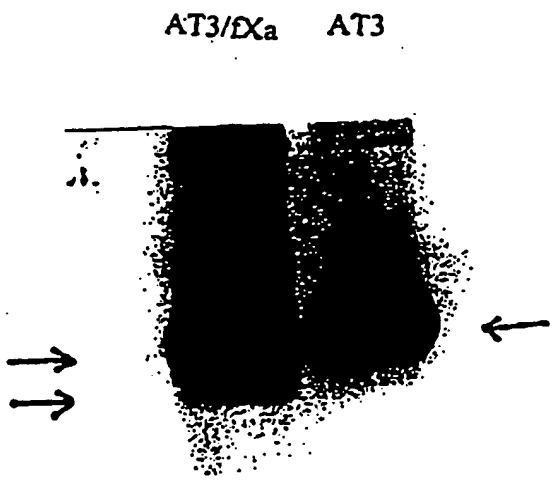


FIGURE 3C

FIGURE 4A

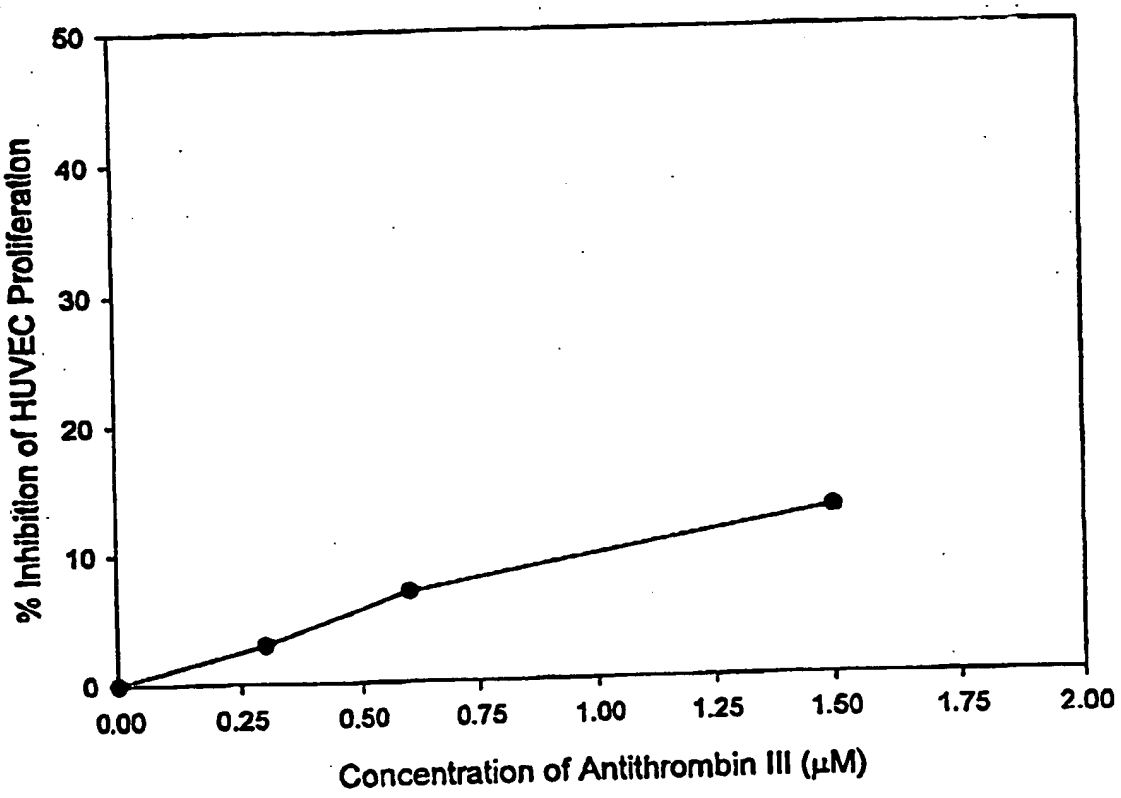
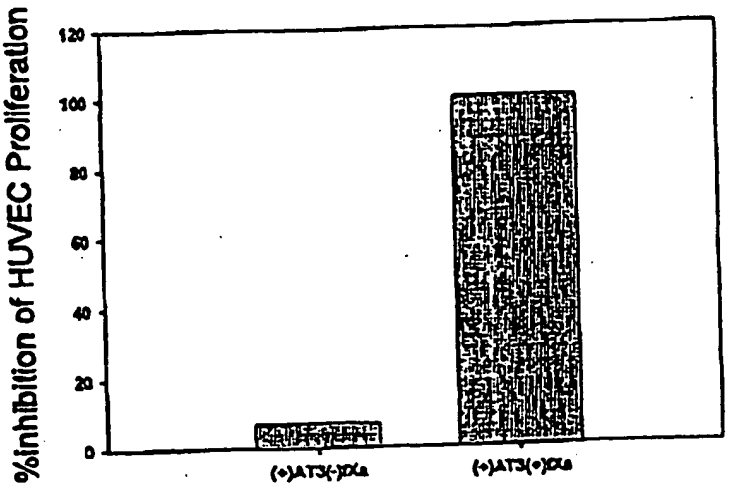


FIGURE 4B



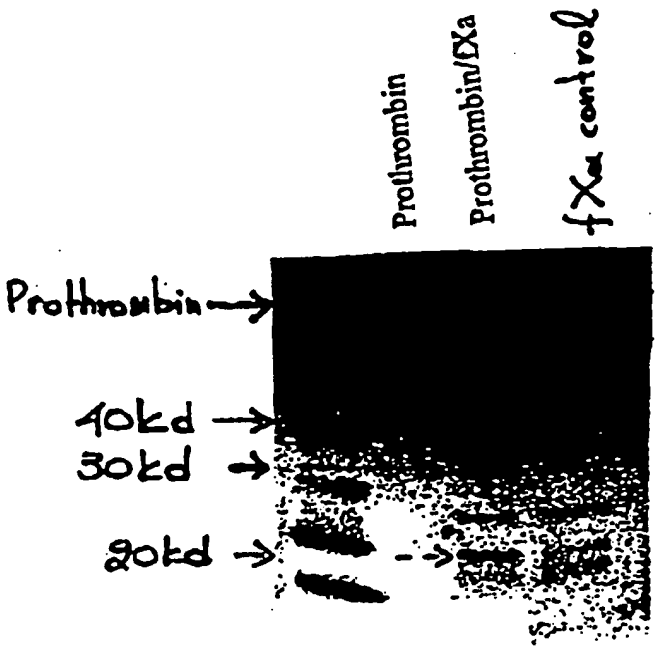

Prothrombin
Prothrombin/fXa
fXa control
Prothrombin
40kd
30kd
20kd

FIGURE 4C

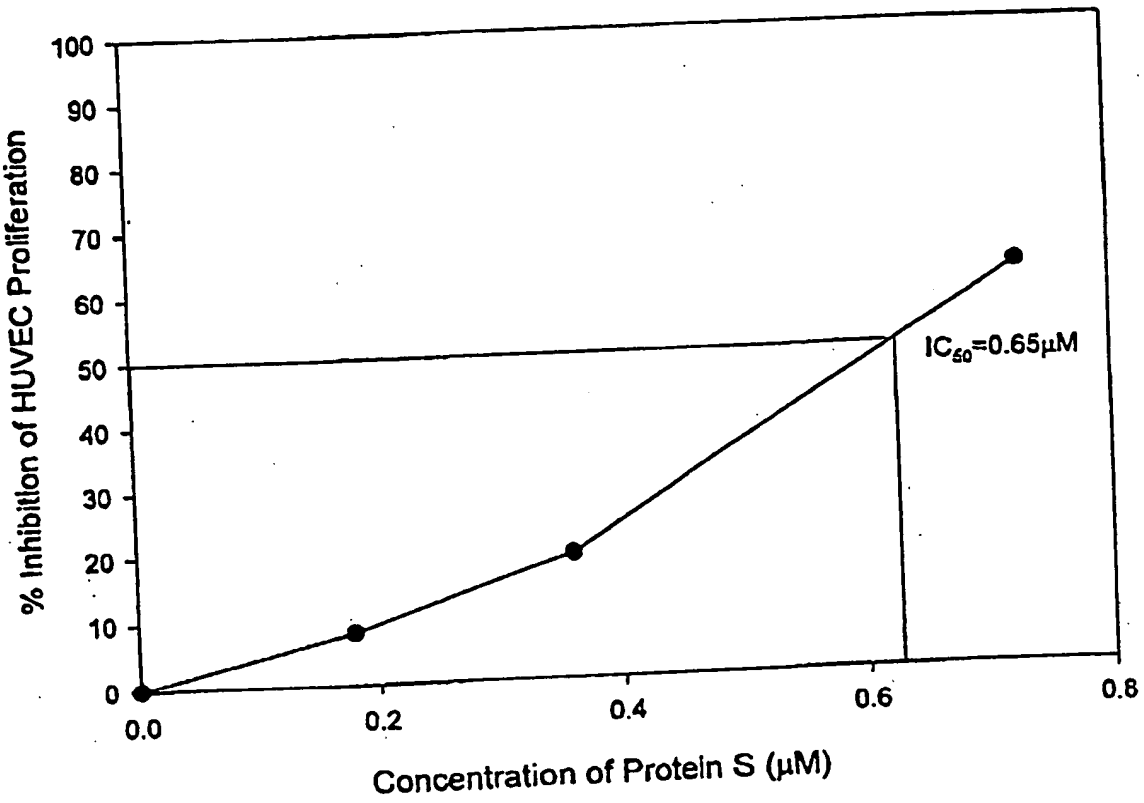

100
90
80
70
60
50
40
30
20
10
0
% Inhibition of HUVEC Proliferation
0.0
0.2
0.4
0.6
0.8
Concentration of Protein S (μM)
IC50=0.65μM

FIGURE 5

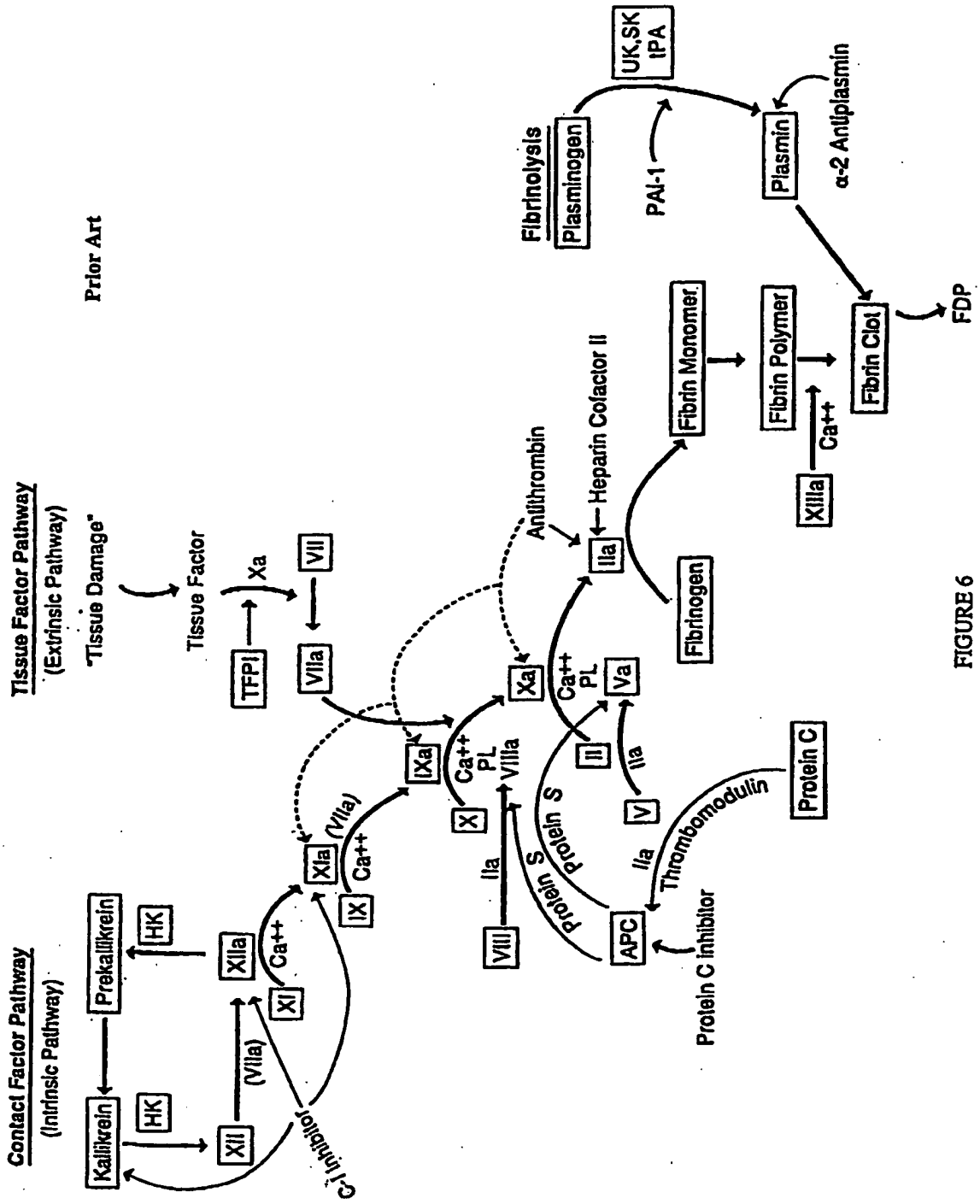

Prior Art
Tissue Factor Pathway
(Extrinsic Pathway)
"Tissue Damage"
Tissue Factor
TFPI
Xa
VII
VIIa
Contact Factor Pathway
(Intrinsic Pathway)
Kallikrein
HK
XII
(VIIa)
Prekallikrein
HK
XIIa
Ca++
XI
C1 Inhibitor
XIa
(VIIa)
Ca++
IX
IXa
Ca++
PL
VIIIa
X
IIa
VIII
Protein S
APC
Protein C inhibitor
IIa
Thrombomodulin
Protein C
V
IIa
Va
II
Ca++
PL
Xa
Antithrombin
Heparin Cofactor II
IIa
Fibrinogen
Fibrin Monomer
Fibrin Polymer
XIIIa
Ca++
Fibrin Clot
FDP
Fibrinolysis
Plasminogen
PAI-1
UK,SK
tPA
Plasmin
α-2 Antiplasmin

FIGURE 6